MEDICAL LIBRARY

VOL. 24 MAY, 1939 NO. 8

MAY 15 1939

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., EDITOR
301 W. Franklin St.
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology
DENNIS E. JACKSON, M.D.
University of Cincinnati, Cincinnati

Tuberculosis
GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Clinical Pathology
W. C. MacCARTY, M.D.
Mayo Clinic, Rochester, Minn.

T. B. MAGATH, M.D.
Mayo Clinic, Rochester, Minn.

Biochemistry
VICTOR C. MYERS, Ph.D.
Western Reserve University, Cleveland

Experimental Medicine
RUSSELL L. HADEN, M.D.
Cleveland Clinic, Cleveland

Immunology
JOHN A. KOLMER, M.D.
Temple University, Philadelphia

ROBERT A. KILDUFFE, M.D.
Atlantic City, N. J.

Internal Medicine
GEORGE HERRMANN, M.D.
University of Texas, Galveston

Bacteriology
M. H. SOULE, Sc.D.
University of Michigan, Ann Arbor

Metabolism
CLIFFORD J. BARBORKA, M.D.
Northwestern University, Chicago

Surgery
DEAN LEWIS, M.D.
Johns Hopkins University, Baltimore

PUBLISHED BY THE C. V. MOSBY COMPANY, 3523-25 PINE BLVD., ST. LOUIS

Copyright 1939 by The C. V. Mosby Company

TABLE OF CONTENTS ON INSIDE COVER

The Journal of Laboratory and Clinical Medicine

CONTENTS FOR MAY, 1939

Clinical and Experimental

Sulfanilamide and Derivatives in the Treatment of Experimental Pneumococcus Infections. John A. Kolmer, M.D., George W. Raiziss, Ph.D., and Anna M. Rule, Philadelphia, Pa. ---- 779

The Determination of Sulfapyridine in Blood. E. G. Schmidt, Ph.D., Baltimore, Md. ---- 795

The Influence of the Water-Soluble Gonadotropic Factor of Pregnancy Urine on the Testes of the Normal Immature and Mature Rat. H. S. Rubinstein, M.D., Ph.D., and A. Abarbanel, A.B., M.D., Baltimore, Md. ---- 799

Excretion of Vitamin C in the Sweat. Irving S. Wright, A.B., M.D., and Elizabeth MacLenathen, B.S., New York, N. Y. ---- 804

The Intradermal Test for Vitamin C Determination. Irving S. Wright, A.B., M.D., and Elizabeth MacLenathen, B.S., New York, N. Y. ---- 806

Potassium Cyanide as an Agent Inhibiting the Oxidation of Vitamin C in Vitro. Irving S. Wright, A.B., M.D., and Elizabeth MacLenathen, B.S., New York, N. Y. ---- 808

Biochemical Observations in Hypoglycemia Induced by Insulin. II. Behavior of Blood Gases in Relation to the Hypoglycemic State. Edward F. Rosenberg, M.D., Rochester, Minn. ---- 809

Biochemical Observations in Hypoglycemia Induced by Insulin. III. Glycorrhachia and Hypoglycemia. Edward F. Rosenberg, M.D., Rochester, Minn. ---- 815

Platelet Studies in Normal Men and Women (Menstruating and Nonmenstruating) and Subjects With Bleeding Disorders. Pearl Lee, M.D., and Betty Nims Erickson, M.A., Detroit, Mich. ---- 821

Action of Sodium Thiocyanate Upon Tissue Oxygen Consumption. Dale G. Friend, M.D., Boston, Mass., and Roger W. Robinson, M.D., Worcester, Mass. ---- 832

Liver Dysfunction as a Possible Causative Factor in Renal Lithiasis. William J. Erickson, A.B., M.D., Philadelphia, Pa. ---- 836

Electrocardiographic Changes Following the Intravenous Administration of Magnesium Sulfate. II. An Experimental Study on Dogs. T. R. Van Dellen, B.S., M.D., and J. Roscoe Miller, M.S., M.D., Chicago, Ill. ---- 840

Laboratory Methods

Sodium Ferrocyanide Excretion in the Estimation of Renal Function. Milton Plotz, M.D., F.A.C.P., and Margarethe Rothenberger, M.D., Brooklyn, N. Y. With the Technical Assistance of Edgar Ferguson and Victor Ginsburg, M.D. ---- 844

A Device for Viewing Precipitin Reactions. Elizabeth M. Kanne, M.A., and Janet R. McCarter, Ph.D., Madison, Wis. ---- 847

A New Blood Culture Media Apparatus. Peter Narvaez, Eldridge, Calif. ---- 849

A Method for the Determination of Erythrocyte Fragility, Using Van Allen Hematocrit Tubes for the Measurement of Changes in Volume of the Cells in Hypotonic Salt Solutions. George M. Guest, M.D., and Mary Wing, M.S., Cincinnati, Ohio ---- 850

A More Rapid Method of Guinea Pig Inoculation for the Diagnosis of Tuberculosis. Carrie I. Woolsey, M.A., Chicago, Ill. ---- 855

A Simple Method for the Aspiration of Bone Marrow. Maurice Morrison, M.D., and A. A. Samwick, B.S., M.D., Brooklyn, N. Y. ---- 858

A Comparative Study of Certain Methods of Measuring Blood Pressure. Joseph B. Cady, M.D., and J. F. Herrick, Ph.D., Rochester, Minn. ---- 861

Rose Bengal Excretion and Hippuric Acid Synthesis Tests of Liver Function: A Comparison. W. P. Stowe, M.D., San Francisco, Calif. ---- 866

A Simple Method of Obtaining Sputum for Pneumococcus Typing. Jack Rom, M.D., Detroit, Mich. ---- 868

A Simple Device for Changing the Light Intensity in the X-Ray Illuminator. Edward J. Van Liere, Ph.D., M.D., and David W. Northup, Ph.D., Morgantown, W. Va. ---- 869

A Manual of Neurohistologic Technique. Oscar A. Turner, M.D., New Haven, Conn. ---- 871

Department of Reviews and Abstracts

Selected Abstracts ---- 888

Vol. 24, No. 8, May, 1939. Journal of Laboratory and Clinical Medicine is published monthly by The C. V. Mosby Company, 3523 Pine Blvd., St. Louis, Mo. Subscription Price: United States, its Possessions, Pan-American Countries, Canada, $8.50 a year; Foreign, $9.50. Entered as Second-Class Matter at Post Office at St. Louis, Mo., under Act of March 3, 1879.

The Journal of Laboratory and Clinical Medicine

VOL. 24 MAY, 1939 No. 8

CLINICAL AND EXPERIMENTAL

SULFANILAMIDE AND DERIVATIVES IN THE TREATMENT OF EXPERIMENTAL PNEUMOCOCCUS INFECTIONS*

JOHN A. KOLMER, M.D., GEORGE W. RAIZISS, PH.D., ANNA M. RULE
PHILADELPHIA, PA.

BECAUSE of the biologic relationship of the pneumococcus to streptococcus, sulfanilamide (para-aminobenzenesulfonamide) and its derivatives have properly commanded considerable interest in relation to the treatment of experimental and human pneumococcus infections.

In 1936 Buttle and Gray[1] reported that sulfanilamide by oral administration had a slight effect upon type I pneumococcus infections of mice inoculated intraperitoneally, but only when very small infective amounts of the organism were employed. Later Buttle and his colleagues[2] found this likewise true of type II pneumococcus.

Rosenthal,[3] however, observed that sulfanilamide in olive oil administered to mice by subcutaneous injection afforded a considerable and encouraging degree of protection against types I, II, and III pneumococci inoculated intraperitoneally. Cooper, Gross, and Mellon[4] also found that the oral administration of the compound prolonged and sometimes saved the lives of mice inoculated subcutaneously with type III pneumococci, and in a subsequent series of investigations Gross and Cooper[5] reported that the compound by oral administration was effective in prolonging and sometimes saving the lives of rats with pneumococcal pneumonia produced by intratracheal injections of types I, II, and III pneumococci suspended in mucin. With type II the results were no more effective than treatment with serum alone, but with type I best results were observed among rats treated simultaneously with sulfanilamide and serum. Schmidt[6] has stated that subcutaneous injections

*From the Research Institute of Cutaneous Medicine, Philadelphia.
Received for publication, October 8, 1938.

of the compound were effective in the treatment of mice inoculated intraperitoneally with type XIV.

We have also observed encouraging results in the treatment of experimental pneumococcus meningitis with sulfanilamide by oral, subcutaneous, and intrathecal administration.[7] Types I, II, and III produced acute meningitis in rabbits by intracisternal inoculation along with an associated septicemia usually fatal in from two to four days. Treatment of 60 animals, begun four hours after inoculation, did not result in the recovery of any, but apparently prolonged the lives of 24 for various periods. The intracisternal inoculation of monkeys with virulent types I, II, and III produced fatal meningitis with septicemia in twenty-four to thirty hours. Treatment of 10 animals with sulfanilamide, begun twenty-four hours after inoculation, resulted in the recovery of one (type III) and apparently prolonged the lives of 7 others.

PURPOSE OF INVESTIGATION

In the present investigation we have sought to determine the influence of sulfanilamide by oral, subcutaneous, intravenous, and a combination of oral and subcutaneous administration upon types I, II, and III pneumococcus infections of rats inoculated intraperitoneally. Our purpose was to select strains of moderate virulence with the hope that these, along with the higher natural resistance of the rat as compared with the mouse, would enable us to administer multiple doses as well as make heart blood cultures.

Our special purpose, however, was to determine the influence of sulfanilamide upon the intradermal pneumococcus lesions of rabbits so frequently accompanied by an associated septicemia produced by the method of Goodner.[8] We have found this experimental lesion very satisfactory not only for chemotherapeutic studies in pneumococcus infections[9, 10] but likewise for chemotherapeutic investigations in streptococcus infections of rabbits[11, 12] because the local lesions of hyperemia, edema, and necrosis not only can be seen and measured daily for clinical purposes, but likewise, and very importantly, can be readily cultured at daily intervals for determining possible local bactericidal effects. Since pneumococcus infections of human beings are predominantly and primarily of the fixed tissues, with secondary invasion and infection of the blood, we have long considered this experimental intradermal pneumococcus "pneumonia" of special value for determining the efficacy of chemical agents not only in the disinfection of the blood, but also, and even more importantly, in the disinfection of the fixed tissues. Kreidler[13] has already reported upon sulfanilamide by oral administration in the treatment of this lesion of rabbits produced by the intradermal injection of 0.1 c.c. of a 1:1,000 dilution of eighteen-hour broth culture of type I pneumococcus, stating that it favorably influenced the septicemia, reduced the fever, and resulted in the recovery of most of the animals.

We have likewise been able to include a study of the acetyl derivative of sulfanilamide as well as 4,4′-diamino-diphenylsulfone first reported upon by Buttle, Stephenson, Smith, and Foster[14] and its acetyl derivative introduced by Fourneau, Trefouel, Nitti, and Bovet.[15] Bauer and Rosenthal[16] have

found 4,4′-diamino-diphenylsulfone and its acetyl derivative more effective than sulfanilamide in pneumococcal infections of mice, but much less marked than against streptococci. Life was prolonged, but few animals permanently survived the pneumococcal infection.

Recently Whitby[17] has reported 2-p-aminobenzenesulfonamide pyridine effective in the treatment of mice infected with types I, II, III, V, VII, and VIII pneumococci, and especially types I, VII, and VIII. Evans and Gailsford[18] report that its use in the treatment of 100 cases of pneumonia resulted in a mortality of 8 per cent as compared with a mortality of 27 per cent in a control group of cases. We have not been able to include this compound in the present investigation, but it is interesting to note in this connection that Kolmer, Brown, Raiziss, Rule, and Clemence[19] have observed particularly encouraging results with two pyridine compounds containing an amino group or an amino group with iodine and especially 2-2′ pyridyl sulfide dihydrobromide in the treatment of intradermal streptococcus infections of rabbits.

SULFANILAMIDE IN TREATMENT OF PNEUMOCOCCUS INFECTIONS OF RATS

In our first experiment 10 white rats, weighing from 100 to 150 gm., were inoculated intraperitoneally with 0.2 c.c. of an eighteen-hour broth culture of type I pneumococcus; 10 additional animals with 0.2 c.c. of a 1:5,000 dilution of type II, and 10 with 0.2 c.c. of 1:600 dilution of type III.

Eight animals of each series were used for treatment with sulfanilamide, the first dose being given four hours after inoculation, and thereafter two doses per day (10 A.M. and 3 P.M.) for four days in succession. Two animals of each series received 0.05 gm. per kg. orally, 2 the same dose subcutaneously,* 2 intravenously, and 2 received 0.025 gm. per kg. orally with 0.025 gm. subcutaneously. The dose administered by all routes corresponded, therefore, to 3.5 gm. per day per 70 kilograms.

TABLE I

EFFECT OF SULFANILAMIDE ON TYPE I PNEUMOCOCCUS INFECTIONS OF RATS*

DOSE PER KG.†				DAYS					
ORALLY	SUBCUT.	INTRAVEN.	ORALLY AND SUBCUTAN.	1	2	3	4	5	6
0.05				–‡	–	D			
0.05				–	–	D			
	0.05			–	–	–	D		
	0.05			–	D				
		0.05		–	–	–	–	–	D
		0.05		–	–	–	D		
			0.025						
			0.025	–	–	D			
Control	–	–	–	–	D				
Control	–	–	–	–	D				

*Inoculated intraperitoneally with 0.2 c.c. of 1:60,000 dilution of eighteen-hour broth culture.

†First dose four hours after inoculation; thereafter twice daily (10 A.M. and 3 P.M.) for four days in succession.

‡– = survival; D = died. All rats succumbing gave positive heart blood cultures.

As shown in Table I, the two controls inoculated with type I succumbed in forty-eight to seventy-two hours. Of the 8 treated animals the lives of only

*0.8 per cent solution in water was employed for all parenteral administrations.

2, or possibly 3, were prolonged by one to three days, all animals ultimately succumbing with positive heart blood cultures.

The two controls inoculated with type II lived for three to five days. Of the 8 treated animals 3 survived the full period of fourteen days' observation and all of these had negative heart blood cultures at this time; the remaining 5 succumbed in two to four days with positive heart blood cultures.

TABLE II

EFFECT OF SULFANILAMIDE ON TYPE II PNEUMOCOCCUS INFECTIONS OF RATS*

DOSE PER KG.†				DAYS											
ORALLY	SUBCUT.	INTRAVEN.	ORALLY AND SUBCUTAN.	1	2	3	4	5	6	7	8	9	10	12	14
0.05				–‡	D										
0.05				–	–	–	–	–	–	–	–	–	–	–	–
	0.05			–	–	D									
	0.05			–	–	–	–	–	–	–	–	–	–	–	–
		0.05		–	–	–	–	–	–	–	–	–	–	–	–
		0.05		–	–	D									
			0.025												
			0.025	–	–	–	D								
			0.025												
			0.025	–	D										
Control	–	–	–	–	–	–	D								
Control	–	–	–	–	–	–	–	–	D						

*Inoculated intraperitoneally with 0.2 c.c. of 1:5000 dilution of eighteen-hour broth culture.

†First dose 4 hours after inoculation; thereafter twice daily (10 A.M. and 3 P.M.) for four days in succession.

‡– = survival; D = died. All rats succumbing gave positive heart blood cultures.

The two controls inoculated with type III succumbed in three days with positive heart blood cultures; of the 8 treated animals 4 survived four to eight days, but all ultimately succumbed with positive blood cultures.

TABLE III

EFFECT OF SULFANILAMIDE ON TYPE III PNEUMOCOCCUS INFECTIONS OF RATS*

DOSAGE PER KG.†				DAYS							
ORALLY	SUBCUT.	INTRAVEN.	ORALLY AND SUBCUTAN.	1	2	3	4	5	6	7	8
0.05				–‡	D						
0.05				–	–	–	–	D			
	0.05			–	–	–	D				
	0.05			–	–	D					
		0.05		–	–	D					
		0.05		–	–	–	–	–	–	–	D
			0.025								
			0.025	–	–	–	–	D			
			0.025								
			0.025	–	–	D					
Control	–	–	–	–	–	D					
Control	–	–	–	–	–	D					

*Inoculated intraperitoneally with 0.2 c.c. of 1:600 dilution of eighteen-hour broth culture.

†First dose four hours after inoculation; thereafter twice daily (10 A.M. and 3 P.M.) for four days in succession.

‡– = survival; D = died. All rats succumbing gave positive heart blood cultures.

This experiment was then repeated with 20 rats inoculated intraperitoneally with 0.2 c.c. of 1:10,000 dilution of eighteen-hour broth culture of type I; 20 inoculated with 0.2 c.c. of 1:600 dilution of type II, and 20 with 0.2 c.c. of 1:600 dilution of type III.

Treatment was started four hours after inoculation, followed by four additional treatments at daily intervals. Four rats of each series were kept as untreated controls, and of the remaining 16 animals, 4 received 0.5 gm. per kg. orally; 4, 0.2 gm. per kg. subcutaneously; 4, 0.5 gm. subcutaneously; and the remaining 4, 0.5 gm. orally with 0.2 gm. subcutaneously per kg. of weight. These amounts corresponded, therefore, to as much as 14 to 49 gm. per 70 kg. per day which were perfectly enormous amounts, but still within the maximum tolerated doses for these animals.

As shown in Table IV, all of the four untreated controls inoculated with type I died in one to two days with positive blood cultures. Of the 16 treated animals the lives of 6 were apparently prolonged for one to two days, but all succumbed with positive heart blood cultures.

TABLE IV

EFFECT OF SULFANILAMIDE ON TYPE I PNEUMOCOCCUS INFECTIONS OF RATS*

DOSAGE PER KG.†			DAYS				
ORALLY	SUBCUT.	ORALLY AND SUBCUTAN.	1	2	3	4	5
0.5			–‡	D			
0.5			–	–	D		
0.5			–	–	–	D	
0.5			–	D			
	0.2		–	D			
	0.2		–	–	D		
	0.2		–	–	D		
	0.2		–	D			
	0.5		–	–	–	D	
	0.5		–	D			
	0.5		–	D			
	0.5		–	–	–	–	D
		0.5					
		0.2	–	D			
		0.5					
		0.2	–	D			
		0.5					
		0.2	–	–	D		
		0.5					
		0.2	–	–	–	–	D
Control	–	–	–	D			
Control	–	–	D				
Control	–	–	D				
Control	–	–	D				

*Inoculated intraperitoneally with 0.2 c.c. of 1:10,000 dilution of eighteen-hour broth culture.

†First dose four hours after inoculation; thereafter daily for four days in succession.

‡– = survival; D = died. All rats succumbing gave positive heart blood cultures.

All of the 4 controls inoculated with type II died in two to six days with positive blood cultures. Of the 16 treated animals, 11 survived for the full period of twenty days' observation at which time all gave negative blood cultures; the remaining 5 died in one to four days with positive blood cultures (Table V).

TABLE V

EFFECT OF SULFANILAMIDE ON TYPE II PNEUMOCOCCUS INFECTIONS OF RATS*

DOSAGE PER KG.†			DAYS															
ORALLY	SUBCUT.	ORALLY AND SUBCUTAN.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	18	20
0.5			–‡	D															
0.5			–	–	–	D													
0.5			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.5			–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.2		–	D															
	0.2		–	–	–	–	D												
	0.2		–	D															
	0.2		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.5		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.5		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.5		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.5		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5																	
		0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5																	
		0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5																	
		0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5																	
		0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control	–	–	–	D															
Control	–	–	–	–	D														
Control	–	–	–	–	D														
Control	–	–	–	–	–	–	–	D											

*Inoculated intraperitoneally with 0.2 c.c. of 1:600 dilution of eighteen-hour broth culture.

†First dose four hours after inoculation; thereafter daily for four days in succession.

‡– = survival; D = died. All rats succumbing gave positive heart blood cultures.

TABLE VI

EFFECT OF SULFANILAMIDE ON TYPE III PNEUMOCOCCUS INFECTIONS OF RATS*

DOSAGE PER KG.†			DAYS															
ORALLY	SUBCUT.	ORALLY AND SUBCUTAN.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	18	20
0.5			–‡	–	–	–	D												
0.5			–	–	D														
0.5			–	–	D														
0.5			–	–	–	–	–	–	–	–	D								
	0.2		–	–	–	–	D												
	0.2		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.2		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.2		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.5		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.5		–	–	–	–	–	–	–	D									
	0.5		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0.5		–	–	–	–	–	–	D										
		0.5																	
		0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5																	
		0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5																	
		0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5																	
		0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control	–	–	–	D															
Control	–	–	–	D															
Control	–	–	–	–	D														
Control	–	–	–	–	–	–	D												

*Inoculated intraperitoneally with 0.2 c.c. of 1:600 dilution of eighteen-hour broth culture.

†First dose four hours after inoculation; thereafter daily for four days in succession.

‡– = survival; D = died. All rats succumbing gave positive heart blood cultures.

All of the 4 controls inoculated with type III succumbed in two to five days with positive blood cultures. Of the remaining 16 treated animals, 9 survived the full period of twenty days' observation at which time the heart blood cultures were sterile; the remaining 7 animals died in three to nine days with positive blood cultures (Table VI).

It would appear, therefore, that sulfanilamide in the amounts administered by these various routes had but very slight influence upon type I pneumococcus peritonitis and septicemia of rats, but that favorable and encouraging results were observed with type II and type III infections. Apparently subcutaneous injections with oral administration gave much better results than oral administration alone.

SULFANILAMIDE IN TREATMENT OF INTRADERMAL PNEUMOCOCCUS INFECTIONS OF RABBITS

In this experiment 10 rabbits were inoculated intradermally (shaven abdominal skin) with 0.2 c.c. of 1:30 dilution of eighteen-hour broth culture of type I pneumococcus, 10 with 0.2 c.c. of 1:30 dilution of type II, and 10 with 0.6 c.c. of 1:30 dilution of type III.

Twenty-four hours later well-developed local lesions of hyperemia and edema were present and, especially with the type II and III strains, at which time the first treatment was given. Subsequently the compound was administered twice daily (10 A.M. and 3 P.M.) for five days in succession, totaling ten treatments in the case of those animals surviving five days or longer.

Sulfanilamide was given orally to 2 rabbits in each series in dose of 0.05 gm. per kg. twice daily; 2 were given the same dose intramuscularly, 2 intravenously, and the remaining 2 of each series received 0.025 gm. orally with 0.025 gm. intramuscularly. These were equivalent to 0.1 gm. daily, corresponding to 7.0 gm. for an adult weighing 70 kg.

Cultures were made of each local lesion daily and heart blood cultures upon the death of the animals.

As shown in Table VII, the two untreated controls inoculated with type I died in two to four days, respectively, with positive lesion and blood cultures. All of the remaining 8 treated animals also succumbed with positive lesion and blood cultures, although the lives of 2 receiving the compound orally were prolonged for about three days.

The two controls inoculated with type II survived for the full period of seven days' observation at which time they showed positive lesion cultures. Of the remaining 8 treated animals 6 likewise survived,, but the lesions of all showed definite clinical improvement and were sterile upon culture (Table VIII).

Both of the untreated controls inoculated with type III also survived the full period of seven days' observation at which time the local lesions were still severe with positive cultures. All of the 8 treated animals also survived and showed definite healing with negative lesion cultures in all except one (Table IX).

TABLE VII

EFFECT OF SULFANILAMIDE UPON INTRADERMAL TYPE I PNEUMOCOCCUS LESIONS OF RABBITS*

DOSE PER KG.†				FIRST DAY‡		SECOND DAY		THIRD DAY		FOURTH DAY		FIFTH DAY		SEVENTH DAY	
ORALLY	INTRAMUS.	INTRAVEN.	ORALLY AND INTRAMUS.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.
0.05				2**	+	3	+	4	+	4	+	4	+	D	+
0.05				2	+	4	+	4	+	4	+	4	+	D	+
	0.05			3	+	D	+								
	0.05			3	+	D	+								
		0.05		3	+	D	+								
		0.05		3	+	4	+	4	+	D	+				
			0.025												
			0.025	2	+	4	+	D	+						
			0.025												
			0.025	2	+	3	+	4	+	D	+				
Control	–	–	–	2	+	3	+	4	+	D	+				
Control	–	–	–	2	+	D	+								

*Inoculated intradermally with 0.2 c.c. of 1:30 dilution of eighteen-hour broth culture.

†Given twice daily (10 A.M. and 3 P.M.) for five days.

‡Twenty-four hours after inoculation, treatment begun.

**Local lesion: 1 = large area of hyperemia; 2 = hyperemia and edema; 3 = severe hemorrhagic lesion; 4 = very severe lesion with necrosis; D = died with positive blood cultures; + = lesion culture positive; – = lesion culture sterile.

TABLE VIII

EFFECT OF SULFANILAMIDE UPON INTRADERMAL TYPE II PNEUMOCOCCUS LESIONS OF RABBITS*

DOSE PER KG.†				FIRST DAY‡		SECOND DAY		THIRD DAY		FOURTH DAY		FIFTH DAY		SEVENTH DAY	
ORALLY	INTRAMUS.	INTRAVEN.	ORALLY AND INTRAMUS.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.
0.05				4**	+	4	+	4	+	4	+	2	–	2	–
0.05				4	+	4	+	4	+	4	+	3	+	D	+
	0.05			4	+	4	+	4	+	2	–	2	–	2	–
	0.05			4	+	D	+								
		0.05		4	+	4	+	4	+	2	–	2	+	2	–
		0.05		4	+	2	–	2	–	2	+	1	+	1	–
			0.025												
			0.025	4	+	3	–	3	+	2	+	2	–	1	–
			0.025												
			0.025	4	+	4	+	3	+	2	+	2	–	1	–
Control	–	–	–	4	+	4	+	4	+	3	+	3	+	3	+
Control	–	–	–	4	+	4	+	4	+	2	+	2	+	2	+

*Inoculated intradermally with 0.2 c.c. of 1:30 dilution of eighteen-hour broth culture.

†Given twice daily (10 A.M. and 3 P.M.) for five days.

‡Twenty-four hours after inoculation; treatment begun.

**Local lesion: 1 = large area of hyperemia; 2 = hyperemia and edema; 3 = severe hemorrhagic lesion; 4 = very severe lesion with necrosis; D = died with positive blood cultures; + = lesion culture positive; – = lesion culture sterile.

TABLE IX

EFFECT OF SULFANILAMIDE UPON INTRADERMAL TYPE III PNEUMOCOCCUS LESIONS OF RABBITS*

DOSE PER KG.†				FIRST DAY‡		SECOND DAY		THIRD DAY		FOURTH DAY		FIFTH DAY		SEVENTH DAY	
ORALLY	INTRAMUS.	INTRAVEN.	ORALLY AND INTRAMUS.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.
0.05				4**	+	4	+	3	+	3	+	2	–	1	–
0.05				3	+	3	+	3	+	2	+	2	–	1	–
	0.05			3	+	3	+	3	+	2	+	1	–	1	+
	0.05			3	+	3	+	2	+	2	+	1	–	1	–
		0.05		4	+	4	+	3	+	2	+	1	–	1	–
		0.05		3	+	3	+	3	+	2	+	1	–		
			0.025												
			0.025	4	+	4	+	4	+	4	+	2	–	1	–
			0.025												
			0.025	3	+	3	+	4	+	3	+	1	–	1	+
Control	–	–	–	4	+	4	+	3	+	3	+	4	+	3	+
Control	–	–	–	3	+	3	+	4	+	4	+	4	+	4	+

*Inoculated intradermally with 0.6 c.c. of 1:30 dilution of eighteen-hour broth culture.

†Given twice daily (10 A.M. and 3 P.M.) for five days.

‡Twenty-four hours after inoculation; treatment begun.

**Local lesion: 1 = large area of hyperemia; 2 = hyperemia and edema; 3 = severe hemorrhagic lesion; 4 = very severe lesion with necrosis; D = died with positive blood cultures; + = lesion culture positive; – = lesion culture sterile.

TABLE X

EFFECT OF SULFANILAMIDE AND DERIVATIVES UPON INTRADERMAL TYPE I PNEUMOCOCCUS LESIONS OF RABBITS*

COMPOUNDS	DOSE PER KG.† (GM.)	FIRST DAY‡		SECOND DAY		THIRD DAY		FOURTH DAY		FIFTH DAY		SEVENTH DAY		TENTH DAY	
		CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.
Sulfanilamide	0.05	2**	+	4	+	4	+	D	+						
	0.125	2	+	4	+	4	+	4	+	D	+				
	0.250	2	+	4	+	4	+	4	+	D	+				
Acetyl derivative of sulfanilamide	0.05	2	+	4	+	D	+								
	0.100	2	+	3	+	3	+	4	+	4	+	D	+		
	0.150	2	+	2	+	4	+	4	+	D	+				
Diamino-diphenyl-sulfone	0.0125	2	+	2	+	D	+								
	0.025	2	+	3	+	4	+	3	+	2	+	2	+	1	–
	0.5	2	+	2	+	4	+	4	+	4	+	D	+		
Diacetyldiamino-diphenylsulfone	0.125	2	+	4	+	D	+								
	0.25	2	+	4	+	4	+	D	+						
	0.5	2	+	D	+										
Controls	–	2	+	4	+	4	+	D	+						
	–	2	+	4	+	3	+	D	+						
	–	2	+	3	+	4	+	D	+						

*Inoculated intradermally with 0.2 c.c. of 1:30 dilution of eighteen-hour broth culture.

†Orally twice daily (10 A.M. and 3 P.M.) for five days.

‡Twenty-four hours after inoculation; treatment begun.

**Local lesion: 1 = large area of hyperemia; 2 = hyperemia and edema; 3 = severe hemorrhagic lesion; 4 = very severe lesion with necrosis; D = died with positive heart blood cultures; + = lesion culture positive; – = lesion culture sterile.

The results have indicated, therefore, that sulfanilamide has a definite curative effect upon intradermal pneumococcus infections of rabbits and especially those produced by types II and III; no essential differences were noted according to the route of administration.

SULFANILAMIDE DERIVATIVES IN TREATMENT OF INTRADERMAL PNEUMOCOCCUS INFECTIONS OF RABBITS

In this experiment 15 rabbits were inoculated intradermally with 0.2 c.c. of eighteen-hour broth culture of type I pneumococcus, 15 with the same dose of type II, and 15 with the same dose of type III.

All three strains produced large local lesions with positive cultures.

Three animals of each series were kept as untreated controls, those inoculated with type I succumbing in four days, and those with type II in five to seven days, with positive lesion and heart blood cultures. The 3 inoculated with type III survived the period of ten days' observation at which time abscesses had developed with positive cultures.

The remaining 12 animals of each series were used for treatment, 3 being given sulfanilamide orally twice a day (10 A.M. and 3 P.M.) for five days in succession, totaling 10 doses in the case of those animals surviving six days or longer after inoculation, since the first dose was given twenty-four hours after inoculation. Of these 3, one received 0.05 gm. (0.1 gm. daily), one 0.125 gm. (0.250 gm. daily), and one 0.250 gm. (0.5 gm. daily) per kilogram. Three animals of each series were treated orally with similar doses of the acetyl derivative of sulfanilamide, 3 with 4,4′-diamino-diphenylsulfone, and 3 with the acetyl derivative of 4,4′-diamino-diphenylsulfone.

As shown in Table X, the 3 untreated controls inoculated with type I pneumococcus succumbed four days after inoculation with positive lesion and heart blood cultures as previously stated. The 3 treated with sulfanilamide died in four to five days, and the 3 treated with its acetyl derivative died in three to seven days. Of the 3 treated with 4,4′-diamino-diphenylsulfone however, one survived the full ten days with marked healing of the local lesion and a negative lesion culture while the remaining 2 died on the third and seventh days, respectively, with positive lesion and blood cultures. All 3 treated with the acetyl derivative of 4,4′-diamino-diphenylsulfone died in two to four days with positive lesion and blood cultures.

The 3 untreated controls inoculated with type II pneumococcus died in five to seven days with positive lesion and blood cultures (Table XI). Of the 3 treated with sulfanilamide one lived the full period of 10 days at which time the local lesion had almost healed with a negative culture; the remaining 2 died five to ten days after inoculation with positive lesion and blood cultures. Of the 3 treated with its acetyl derivative, 2 lived the full period of ten days, at which time some healing of the local lesion had occurred, but both showing positive lesion cultures.

All 3 treated with 4.4′-diamino-diphenylsulfone survived for ten days, at which time the lesions showed considerable healing, but still yielding positive cultures.

TABLE XI

EFFECT OF SULFANILAMIDE AND DERIVATIVES UPON INTRADERMAL TYPE II PNEUMOCOCCUS LESIONS OF RABBITS*

COMPOUNDS	DOSE PER KG.† (GM.)	FIRST DAY‡		SECOND DAY		THIRD DAY		FOURTH DAY		FIFTH DAY		SEVENTH DAY		TENTH DAY	
		CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.
Sulfanilamide	0.05	4**	+	4	+	3	+	3	+	2	–	1	–	1	–
	0.125	4	+	4	+	3	+	2	+	D	+				
	0.250	4	+	4	+	3	+	3	+	2	+	2	+	D	+
Acetyl derivative of sulfanilamide	0.05	4	+	4	+	4	+	4	+	4	+	2	+	2	+
	0.100	4	+	4	+	4	+	4	+	4	+	3	+	2	+
	0.150*	4	+	4	+	4	+	3	+	D	+				
Diamino-diphenylsulfone	0.0125	4	+	4	+	4	+	4	+	2	+	2	+	2	+
	0.025	4	+	4	+	4	+	4	+	2	+	1	+	1	+
	0.5	4	+	4	+	4	+	4	+	3	+	1	+	1	+
Diacetyl-diamino-diphenylsulfone	0.125	4	+	4	+	4	+	4	+	2	+	2	+	1	+
	0.25	4	+	4	+	4	+	3	+	3	+	2	+	1	+
	0.5	4	+	4	+	4	+	4	+	3	+	2	+	2	+
Controls	–	4	+	4	+	4	+	4	+	D	+				
	–	4	+	4	+	4	+	4	+	4	+	D	+		
	–	4	+	4	+	4	+	4	+	D	+				

*Inoculated intradermally with 0.2 c.c. of 1:30 dilution of eighteen-hour broth culture.

†Orally twice daily (10 A.M. and 3 P.M.) for five days.

‡Twenty-four hours after inoculation; treatment begun.

**Local lesion: 1 = large area of hyperemia; 2 = hyperemia and edema; 3 = severe hemorrhagic lesion; 4 = very severe lesion with necrosis; D = died with positive heart blood cultures; + = lesion culture positive; – = lesion culture sterile.

TABLE XII

EFFECT OF SULFANILAMIDE AND DERIVATIVES UPON INTRADERMAL TYPE III PNEUMOCOCCUS LESIONS OF RABBITS*

COMPOUNDS	DOSE PER KG.† (GM.)	FIRST DAY‡		SECOND DAY		THIRD DAY		FOURTH DAY		FIFTH DAY		SEVENTH DAY		TENTH DAY	
		CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.	CLIN.	CULT.
Sulfanilamide	0.05	2**	+	3	+	4	+	2	+	1	–	1	–	–	–
	0.125	3	+	4	+	1	+	1	+	1	–	1	–	–	–
	0.250	3	+	3	+	2	+	1	–	1	–	1	–	–	–
Acetyl derivative of sulfanilamide	0.05	3	+	3	+	4	+	2	+	1	+	1	+	1	+
	0.100	2	+	4	+	2	+	2	–	1	–	Abs.	–	Abs.	–
	0.150	3	+	4	+	4	+	2	+	2	+	2	+	D	+
Diamino-diphenyl-sulfone	0.0125	3	+	4	+	2	+	2	–	1	–	1	–	1	–
	0.25	3	+	2	+	2	+	1	–	1	–	1	–	–	–
	0.5	3	+	2	+	2	+	1	–	1	–	1	–	D	+
Diacetyl-diamino-diphenylsulfone	0.0125	3	+	4	+	3	+	2	+	2	+	1	–	1	–
	0.25	2	+	4	+	2	+	2	+	2	–	1	–	1	–
	0.5	3	+	4	+	2	+	2	–	1	–	1	–	1	–
Controls	–	3	+	4	+	4	+	3	+	3	+	Abs.	+	Abs.	+
	–	3	+	4	+	4	+	2	+	Abs.	+	Abs.	+	Abs.	+
	–	3	+	4	+	2	+	Abs.	+	Abs.	+	Abs.	+	Abs.	+

*Inoculated intradermally with 0.2 c.c. of 1:30 dilution of eighteen-hour broth culture.

†Orally twice daily (10 A.M. and 3 P.M.) for five days.

‡Twenty-four hours after inoculation; treatment begun.

**Local lesion: 1 = large area of hyperemia; 2 = hyperemia and edema; 3 = severe hemorrhagic lesion; 4 = very severe lesion with necrosis; D = died with positive heart blood cultures; + = lesion culture positive; – = lesion culture sterile.

The 3 treated with the acetyl derivative of 4,4′-diamino-diphenylsulfone also survived the full ten days and likewise with considerable healing of the local lesions, but all showing positive lesion cultures.

As previously stated, all 3 untreated controls inoculated with type III pneumococcus survived the period of ten days, but abscesses developed with positive cultures (Table XII).

All 3 treated with sulfanilamide likewise survived with complete healing of the local lesions and negative cultures. Of the 3 treated with its acetyl derivative one died on the tenth day with a positive lesion and blood culture, while the remaining 2 survived, one with a negative lesion culture.

Of the 3 treated with 4,4′-diamino-diphenylsulfone 2 survived ten days with complete or almost complete healing with negative lesion cultures, while the remaining animal died on the tenth day with a local lesion well healed but with a positive lesion and blood culture. All 3 treated with its acetyl derivative lived the full ten days with almost complete healing of the local lesions along with negative lesion cultures.

CONCLUSIONS

1. Sulfanilamide by oral, subcutaneous, intravenous, and combined oral and subcutaneous administration was only very slightly effective in the treatment of type I pneumococcus peritonitis and septicemia of rats. Much better results were observed in types II and III infections.

2. Sulfanilamide by oral, intramuscular, intravenous, and combined oral and intramuscular administration was only slightly effective in the treatment of type I intradermal pneumococcus infections and septicemia of rabbits. Much better results were observed with type II and type III infections.

3. The acetyl derivative of sulfanilamide was less effective than sulfanilamide itself in the treatment of types I, II, and III intradermal pneumococcus infections and septicemia of rabbits by the four routes of administration.

4. 4,4′-Diamino-diphenylsulfone was more effective than sulfanilamide in the treatment of type I and type II intradermal pneumococcus infections of rabbits by the four routes of administration, but probably slightly less effective in the treatment of type III infections.

5. The diacetyl derivative of 4,4′-diamino-diphenylsulfone was less effective than sulfanilamide and diamino-diphenylsulfone in the treatment of type I and type II intradermal pneumococcus infections of rabbits with septicemia by the four routes of administration, but about equal to these compounds in the treatment of type III infections.

REFERENCES

1. Buttle, G. A. H., and Gray, W. H.: Protection of Mice Against Streptococcal and Other Infections by p-Aminobenzenesulfonamide and Related Substances, Lancet **1:** 1286, 1936.
2. Buttle, G. A. H., Parrish, H. J., McLeod, M., and Stephenson, D.: The Chemotherapy of Typhoid and Some Other Non-Streptococcal Infections in Mice, Lancet **1:** 681, 1937.
3. Rosenthal, S. M.: Chemotherapy of Experimental Pneumococcus Infections, Pub. Health Rep. **52:** 48, 662, and 685, 1937.
4. Cooper, F. B., Gross, P., and Mellon, R. R.: Action of p-Aminobenzenesulfonamide on Type III Pneumococcus Infections in Mice, Proc. Soc. Exper. Biol. & Med. **36:** 148, 1937.

5. Gross, P., and Cooper, F. B.: Efficacy of p-Aminobenzenesulfonamide in Experimental Type III Pneumococcus Pneumonias of Rats, Proc. Soc. Exper. Biol. & Med. **36**: 225, 535, 678, and 774, 1937.
6. Schmidt, L. H.: Use of Sulfanilamide in the Treatment of Type XIV Pneumococcus Infections in Mice, Proc. Soc. Exper. Biol. & Med. **37**: 205, 1937.
7. Kolmer, J. A., Rule, A. M., and Werner, M. L.: Sulfanilamide in the Treatment of Experimental Streptococcic and Pneumococcic Meningitis, Arch. Otolaryng. **27**: 519, 1938.
8. Goodner, K.: Experimental Intradermal Pneumococcus Infection in Rabbits, J. Exper. Med. **48**: 1, 1928; Ibid. **54**: 817, 1931.
9. Kolmer, J. A., and Rule, A. M.: Goodner's Intradermal Pneumococcus Infection of Rabbits, J. Infect. Dis. **57**: 46, 1935.
10. Kolmer, J. A., and Rule, A. M.: Chemotherapy of Intradermal Pneumococcus Infection of Rabbits. Effects of Optochin and Other Quinine Compounds, Proc. Soc. Exper. Biol. & Med. **32**: 89, 1934.
11. Kolmer, J. A., and Rule, A. M.: Experimental Streptococcus Infections in Rabbits for Therapeutic Investigations, J. Lab. & Clin. Med. **22**: 1097, 1937.
12. Kolmer, J. A., Brown, H. B., Rule, A. M., and Werner, M. F.: Toxicity, Therapeutic Activity, and Mode of Action of Sulfanilamide in Experimental Streptococcus Infections of Rabbits, J. Lab. & Clin. Med. **24**: 164, 1938.
13. Kreidler, W. A.: Treatment of Pneumococcal Infections in Rabbits With Sulfanilamide, Proc. Soc. Exper. Biol. & Med. **37**: 146, 1937.
14. Buttle, G. A., Stephenson, D., Smith, S., and Foster, G. E.: Treatment of Streptococcal Infections in Mice With 4:4′ Diaminodiphenylsulfone, Lancet **1**: 1331, 1937.
15. Fourneau, E., Trefouel, J., Nitti, F., and Bovet, D.: Action Antistreptococcique der dérivés sulfures organiques, Compt. rend. Acad. d. sc. **204**: 1763, 1937; Ibid. **205**: 299, 1937.
16. Bauer, H., and Rosenthal, S. M.: Some New Sulphur Compounds Active Against Bacterial Infections, Pub. Health Rep. **53**: 40, 1938.
17. Whitby, L. E. H.: Chemotherapy of Pneumococcal and Other Infections With 2-p-Aminobenzenesulfonamido Pyridine, Lancet **1**: 1210, 1938.
18. Evans, G. M., and Gailsford, W. F.: Treatment of Pneumonia With 2-p-Aminobenzenesulfonamido Pyridine, Lancet **2**: 14, 1938.
19. Kolmer, J. A., Brown, H., Raiziss, G. W., Rule, A. M., and Clemence, L. W.: The Chemotherapy of Experimental Streptococcus Infections of Rabbits With Special Reference to Pyridine Compounds and Prontosil Soluble, J. Pharmacol. & Exper. Therap. **61**: 253, 1937.

THE DETERMINATION OF SULFAPYRIDINE IN BLOOD*

E. G. Schmidt, Ph.D., Baltimore, Md.

RECENT experimental evidence indicates that 2 (p-aminobenzenesulfonamido)-pyridine[1-3] or sulfapyridine[4] is a more promising chemotherapeutic agent in pneumonia therapy than sulfanilamide itself. Intelligent treatment with this drug will require a knowledge of its concentration in the blood.[5-7] In the present paper data are given on the determination of sulfapyridine in the blood by means of three methods which have been used successfully for sulfanilamide analysis: the Marshall[8-11] and the Proom[12-13] diazotization procedures, and the naphthoquinonesulfonic acid method[14-17] of the author which can be performed on routine blood filtrates.

METHODS OF ANALYSIS AND RECOVERY OF ADDED SULFAPYRIDINE

The general plan of approach was the same as that used by the author in previous work on sulfanilamide analysis.[14-16] To 18 c.c. portions of pooled, oxalated, human blood were added 2 c.c. quantities of stock sulfapyridine†

*From the Department of Biological Chemistry, University of Maryland, School of Medicine, Baltimore.

†Merck and Co., Inc., kindly furnished the sulfapyridine used in these experiments.

solutions of such strengths as to yield a series of bloods containing 1 to 20 mg. sulfapyridine per 100 c.c. The stock solution was prepared by dissolving 1 gm. of the drug in hot distilled water. This solution was cooled, then diluted to 1000 c.c. Each cubic centimeter contained 1 mg. sulfapyridine. After a few days some of the compound would crystallize; when this occurred the solution was discarded. The various standards for analysis were prepared from a stock solution which contained 0.1 mg. per c.c. and which remained clear and stable even in the cold.

In the Marshall method exactly 3 c.c. quantities of blood were laked with 21 c.c. of 0.05 per cent saponin solution and then deproteinized with 6 c.c. of a 20 per cent p-toluenesulfonic acid solution. To 10 c.c. of filtrate was added 1 c.c. of freshly prepared 0.1 per cent sodium nitrite, followed by 1 c.c. of the phosphate-sulfamate solution,[11] and then by 5 c.c. of alcoholic, heat-activated[10] dimethyl-alpha-naphthylamine. Appropriate standards were treated in a similar manner, and comparisons were made in the colorimeter at the end of ten minutes, although the color would remain stable for at least an hour. In the Proom modification 3 c.c. of blood were laked with 21 c.c. of water, followed by deproteinization with 6 c.c. of 15 per cent trichloracetic acid. Ten cubic centimeters of this filtrate and an equal volume of the various standards were treated as in the Marshall procedure.[11] In the naphthoquinonesulfonic acid method 3 c.c. quantities of blood were laked with 24 c.c. of 0.12 N sulfuric acid, followed by 3 c.c. of 10 per cent sodium tungstate solution. A drop of 0.1 N hydrochloric acid was added to 10 c.c. portions of each filtrate and standards, followed by 1 c.c. of 0.05 per cent sodium-beta-naphthoquinone-4-sulfonate (prepared from a 0.5 per cent solution, which remains stable for at least a month in a cold, dark place). The tubes were placed in the dark for an hour, after which the orange colored solutions were matched in the colorimeter. Since the reagent itself is colored, correct values can be obtained only if the unknown and the standard used for comparison are nearly alike.

TABLE I

RECOVERY OF SULFAPYRIDINE ADDED TO BLOOD BY VARIOUS METHODS OF ANALYSIS

NUMBER OF DETERMINATIONS	SULFAPYRIDINE ADDED TO BLOOD MG./100 C.C.	RECOVERY BY											
		MARSHALL METHOD				PROOM-MARSHALL METHOD				NAPHTHOQUINONE-SULFONIC ACID METHOD			
		MG./100 C.C. BLOOD			PER CENT AVERAGE	MG./100 C.C. BLOOD			PER CENT AVERAGE	MG./100 C.C. BLOOD			PER CENT AVERAGE
		MAX.	MIN.	AVG.		MAX.	MIN.	AVG.		MAX.	MIN.	AVG.	
1	20.0			19.7	98.5			18.5	92.5			16.0	80.0
4	10.0	10.0	9.8	9.87	98.7	9.5	9.1	9.3	93.0	8.2	7.9	8.1	81.0
4	5.0	5.1	4.8	4.9	98.0	4.8	4.6	4.65	93.0	4.3	3.8	4.1	82.0
5	2.5	2.6	2.4	2.5	100.0	2.27	2.17	2.23	89.2	2.2	2.0	2.05	82.0
1	1.0			0.98	98.0			0.94	94.0			0.84	84.0
		Average per cent			98.6	Average per cent			92.3	Average per cent			81.8

The data in Table I indicate that excellent recovery (98 to 100 per cent) of added sulfapyridine was obtained with the Marshall method,[11] but that quantitative recovery was not secured on the trichloracetic acid filtrates and that

8 per cent of the added sulfapyridine was adsorbed by the precipitated proteins. This fact was confirmed in the following manner: Ten cubic centimeters of sulfapyridine solution containing 1 mg. of the drug were diluted to 100 c.c. with control trichloracetic acid blood filtrate. Another 10 c.c. portion was diluted to the same volume with water containing 18 c.c. of 15 per cent trichloracetic acid per 100 c.c. Ten cubic centimeter quantities of each of these solutions gave exactly the same amount of color after the addition of the proper reagents. This differs from the sulfanilamide experiments wherein quantitative recovery was always obtained from the trichloracetic acid filtrates also. The data also show that 18 per cent of the added sulfapyridine was adsorbed by the protein-tungstate precipitate, as compared with 10 per cent in case of sulfanilamide reported in a previous paper.[16] Since these losses are consistent, however, regardless of the concentration of sulfapyridine in the blood, correct values can be secured by dividing the obtained values by the factors 0.92 and 0.82, respectively.

TABLE II

COMPARATIVE ANALYSIS OF THE BLOOD OF PATIENTS UNDERGOING SULFAPYRIDINE THERAPY*

MARSHALL METHOD ON P-TOLUENESULFONIC ACID FILTRATES	(A) PROOM-MARSHALL METHOD ON TRICHLORACETIC ACID FILTRATES	(A) / 0.92	(B) NAPHTHOQUINONE-SULFONIC ACID METHOD ON TUNGSTIC ACID FILTRATES	(B) / 0.82
16.0			13.0	15.9
12.0	11.5	12.5	9.6	11.7
10.0	9.0	9.8	8.2	10.0
9.1	8.3	9.0	7.2	8.8
8.6	7.8	8.5	7.2	8.8
5.7	5.3	5.8	5.0	6.1
5.3	5.0	5.4	4.3	5.2
4.6	4.2	4.6	3.4	4.1
3.1	2.7	2.9	2.5	3.0
2.2	1.9	2.1	1.8	2.2
1.5	1.3	1.4	1.4	1.7
0.8	0.7	0.8	0.7	0.9

*The values are given as mg. of sulfapyridine per 100 c.c. of blood.

In Table II data are given on a series of bloods secured from patients undergoing sulfapyridine therapy. Again it is obvious that the values given by the Marshall procedure[11] on the trichloracetic acid filtrates are slightly lower than those given by the same method on the p-toluenesulfonic acid filtrates. When these lower values are corrected for sulfapyridine loss, incidental to deproteinization, by the factor 0.92, values are secured which check well with the higher and more correct ones given by the regular Marshall method. The findings by the naphthoquinonesulfonic acid method are likewise low. When these findings are divided by the correction factor 0.82, in this case, values are secured which also check closely with those given by the Marshall procedure.

CONCLUSIONS AND SUMMARY

The sulfapyridine concentration of blood can be accurately determined by the recent Marshall method on p-toluenesulfonic acid filtrates. On trichloracetic acid filtrates, however, this method gives values which are low by 8 per cent. The naphthoquinonesulfonic acid method on tungstic acid filtrates gives results

which are low by 18 per cent. However, correct values can be obtained in each case by the use of proper correction factors. The regular Marshall procedure has the advantage of greater color production, better proportionality, and probably greater accuracy. However, the naphthoquinonesulfonic acid method seems to fit in better with the routine hospital work. Thus, it is carried out on the regular Folin-Wu tungstic acid blood filtrates, requires fewer and cheaper reagents, and a shorter working time.

REFERENCES

1. Whitby, L. E. H.: Chemotherapy of Pneumococcal and Other Infections With 2 (p-Aminobenzenesulphonamido) pyridine, Lancet **1**: 1210, 1938.
2. Evans, G. M., and Gaisford, W. F.: Treatment of Pneumonia With 2 (p-Aminobenzenesulphonamido) pyridine, Lancet **2**: 14, 1938.
3. Paris Foreign Letter, Action of Sulfanilamide-Pyridine on the Pneumococcus, J. A. M. A. **111**: 2404, 1938.
4. Report of the Council, J. A. M. A. **112**: 49, 1939.
5. Hobson, F. G., and MacQuaide, D. H. G.: Treatment of Meningococcal Meningitis With 2-Sulphanilyl-Amidopyridine (M. & B. 693), Lancet **2**: 1213, 1938.
6. Maegraith, B. G., and Vollum, R. L.: The Bacteriostatic Effects of Sulphonamide-P, Soluseptasine and M. & B. 693, Brit. M. J. **2**: 985, 1938.
7. Mayer, R. L.: Activité de l'alpha (p-aminophényl-sulfamido)-pyridine dans les infections colibacillaire et staphlococcique de lat souris, Compt. rend. Soc. de biol. **129**: 480, 1938.
8. Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C.: p-Aminobenzenesulfonamide, J. A. M. A. **108**: 953, 1937.
9. Marshall, E. K., Jr.: Determination of Sulfanilamide in Blood and Urine, Proc. Soc. Exper. Biol. & Med. **36**: 422, 1937.
10. Marshall, E. K., Jr.: Determination of Sulfanilamide in Blood and Urine, J. Biol. Chem. **122**: 263, 1937.
11. Marshall, E. K., Jr., and Litchfield, J. T., Jr.: The Determination of Sulfanilamide, Science **88**: 85, 1938.
12. Proom, H.: Estimation of Sulfanilamide in Blood and Other Body Fluids, Lancet **1**: 260, 1938.
13. Kamlet, J.: The Determination of Sulfanilamide in Blood, J. LAB. & CLIN. MED. 23: 1101, 1938.
14. Schmidt, E. G.: The Determination of Sulfanilamide in Tungstic Acid Blood Filtrates by Means of Sodium-β-Naphthoquinone-4-Sulfonate, J. Biol. Chem. **122**: 757, 1938.
15. Schmidt, E. G.: The Determination of Sulfanilamide in Cerebrospinal Fluid by Means of Sodium-Beta-Naphthoquinone-4-Sulfonate, J. LAB. & CLIN. MED. **23**: 648, 1938.
16. Schmidt, E. G.: Further Observations on the Determination of Sulfanilamide in Blood. J. LAB. & CLIN. MED. In Press.

THE INFLUENCE OF THE WATER-SOLUBLE GONADOTROPIC FACTOR OF PREGNANCY URINE ON THE TESTES OF THE NORMAL IMMATURE AND MATURE RAT*

H. S. Rubinstein, M.D., Ph.D., and A. Abarbanel, A.B., M.D.
Baltimore, Md.

THE classic work of Zondek (1926), Smith and Engle (1927), Evans and others (1933) has shown that the anterior pituitary gland controls the gonads. Zondek (1931) has shown that in pregnancy enormous amounts of pituitary-like hormone are excreted in the urine. This hormone he has called prolan. Since this earlier work, a huge literature has accumulated on the effects of prolan on the gonads. While most of these reports concern themselves with the effects upon hypophysectomized animals, relatively few critical studies have been carried out on the normal subject. Furthermore, reports dealing with the effects of pregnancy urine extract (prolan) upon the male gonad are at present quite conflicting. For example, some deny its effect upon the germinal epithelium (Engle, 1932; Molien, D'Amour, and Gustavson, 1933; Robson and Taylor, 1933; Collip, 1934), while others maintain that spermatogenesis is either accelerated or increased (Brouha and Simonnet, 1929; Borst, Doderlein, and Gastimorovic, 1930; Krause, 1930; Boeters, 1931). Moreover, Brosius and Schaffer (1933) and Lloyd (1936) have reported spermatogenic stimulation in the human adult.

In view of these conflicting reports, some of which stood in contradiction to the results of one of us (Rubinstein, 1938), in producing an increased spermatozoal output in normal human males with this substance, we decided to reinvestigate the problem.

For this purpose, 40 albino male rats of Wistar Institute stock from thirty to one hundred days old were studied. One-half of these (20) served as controls. Both groups were further subdivided into those older than forty-five days (age of normal testicular descent—Donaldson, 1924) and those younger. All animals were kept on a diet of Purina dog chow daily, with green vegetables added twice weekly. Water was constantly present. The test animals received daily intraperitoneal injections of the water-soluble gonadotropic factor of pregnancy urine to a total of 25 rat units for the ten days. The animals were sacrificed twenty-four hours after the last injection. Body and testicular weights were recorded, and the gonads were studied grossly and microscopically.

RESULTS

The extract in the doses used had no effect upon the body weights. Premature testicular descent, as previously reported (Rubinstein, 1934), was noted

*From the Laboratory for Research, Surgical Division, Sinai Hospital, Baltimore.
Received for publication, October 4, 1938.

in all treated animals. In addition, it led to significant increase in testicular weights in both mature and immature groups as shown in Table I.

TABLE I

BODY WEIGHTS AND TESTICULAR WEIGHTS OF ALL ANIMALS

	BODY WEIGHT				TESTICULAR WEIGHT			
	TEST ANIMALS	CONTROLS	DIFFERENCE	C. R.*	TEST ANIMALS	CONTROLS	DIFFERENCE	C. R.
Immature	48.0 ± 1.93	52 ± 2.54	−4 ± 3.2	1.2	0.352 ± 0.004	0.308 ± 0.007	0.044 ± 0.007	6.3
Mature	130.0 ± 5.82	122 ± 4.59	8 ± 5.8	1.4	1.996 ± 0.084	1.701 ± 0.043	0.295 ± 0.094	3.1

*C. R. indicates the ratio of difference. Differences are considered as probably significant only if this ratio is 3 or more.

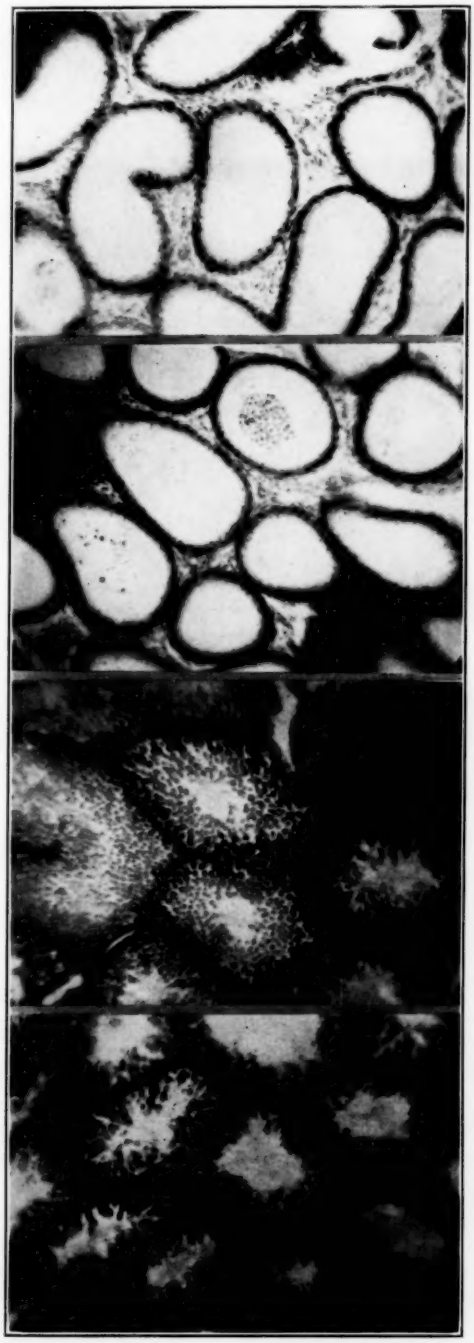
Histologically, the testes of the immature treated animals (Fig. 1*A*) showed a marked increase in interstitial tissue, a moderate hyperemia, a definite increase in the number of spermatogonia and spermatocytes, and an increased number of mitotic figures. Spermatids were occasionally present, but mature spermatozoa could not be seen. In contrast to this the immature control testes (Fig. 1*B*) showed interstitial tissue which was very scant and mitotic figures which were considerably less in number. Mature sperm were also absent here. In the epididymis of the immature test animals (Fig. 1*C*) could be seen clumps of immature sperm consisting of spermatocytes which were often in active mitosis. The epithelium was definitely secretory in character. No such cell groups could be seen in the normal immature control epididymis (Fig. 1*D*).

The testes of the adult test animals showed a marked hyperemia and an increase in interstitial tissue. In addition, there was a slight increase of mitotic figures among the spermatogonia and spermatocytes as compared to the controls (Fig. 2*A* and *B*). Since the mature group showed mature spermatozoa in both test and control rats, no striking difference could be observed in their epididymides.

DISCUSSION

Practically all reports agree that the water-soluble gonadotropic factor of pregnancy urine (P. U. extract) causes a marked increase in the interstitial tissue of mature and immature rats. Our findings in this respect are merely corroborative. In addition to this effect the germinal epithelium in our animals also showed evidence of germinal cell stimulation resulting in a marked proliferation of spermatogonia and spermatocytes. Although this effect was quite noticeable in younger animals, no mature sperm were seen in this group.

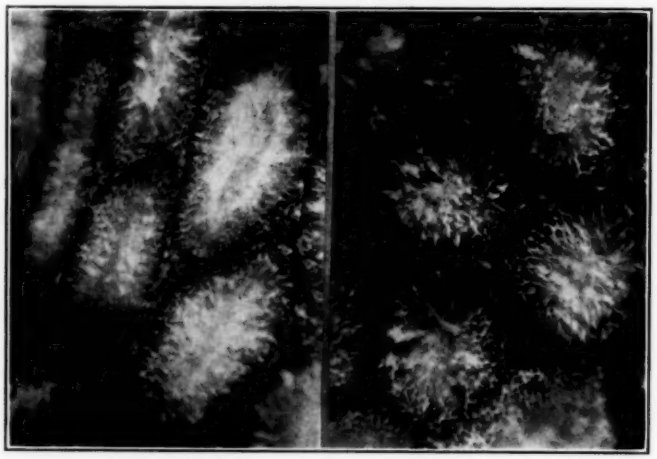
The mechanism of the action of pregnancy urine extract upon the germinal epithelium of the testis is probably secondary to the liberation of androgenic substance through its action upon the interstitial cells. One is led to this explanation for the following reasons: First, the administration of androgens alone will lead to precocious testicular descent without evidence of interstitial cell involvement. Hamilton (1937) has shown this to be true in the monkey, and the same result has been obtained in this laboratory in the rat. Secondly, growth and maturation of the accessory sex organs as well as maintenance results



A. *B.* *C.* *D.*

Fig. 1.—Testes and epididymis of immature test and control rats. *A*, Testis of test animal showing increased interstitial tissue and mitotic figures. *B*, Control testis showing very little interstitial tissue and predominantly inactive germinal cells. *C*, Test epididymis showing clumps of testicular germ cells within the lumina. *D*, Empty lumina of the epididymis of the test animal.

when androgens are administered to the castrate or immature animal. Thirdly, testosterone propionate will bring about proliferative changes of the germinal epithelium in the normal immature animal as well as in the hypophysectomized rat (Nelson and Gallagher, 1936). Correlative evidence is adduced by the fact that this androgen as well as pregnancy urine extract results in an increased sperm count in normal human adults (Rubinstein and Kurland).



A. *B.*

Fig. 2.—Testes of mature animals. *A*, Prolan treated animals showing increased interstitial tissue. *B*, Control.

Because the results enumerated above through the administration of male hormone (testosterone) can also be obtained with pregnancy urine extract which predominantly stimulates the interstitial tissue, and because it is now known that the interstitial tissue secretes the androgenic hormone, one may safely assume that the effects of pregnancy extract upon the germinal epithelium result secondarily to the liberated androgen.

CONCLUSIONS

The water-soluble gonadotropic factor of pregnancy urine injected into normal mature and immature rats leads to precocious testicular descent, significantly enlarged testes (by weight), and increased interstitial tissue. While this hormone stimulates germinal cell proliferation, it does not hasten maturation.

Its mode of action is discussed and it is assumed that while its effect upon the interstitial tissue is direct, its germinal proliferative activity is secondary to testicular androgenic influence.

REFERENCES

1. Boeters, H.: Das Hypophysenvorderlappenhormon (Prolan) und die mannliche Keimdruse, Virchows Arch. f. path. Anat. **280:** 215, 1931.
2. Borst, M., Doderlein, A., and Gastimorovic, D.: Ueber die Einwirkung des Hypophysenvorderlappenhormons (Prolan) auf juvenile männliche Mäuse, München. med. Wchnschr. **77:** 473, 1536, 1930.
3. Brosius, W. L., and Schaffer, R. L.: Spermatogenesis Following Therapy With the Gonad Stimulating Extract From Urine of Pregnancy, J. A. M. A. **101:** 1227, 1933.

4. Brouha, L., and Simonnet, H.: Hormone anterhypophysaire et tractus genitale male, Ann. de physiol. **5**: 562, 1929.
5. Collip, J. B.: Some Recent Advances in the Physiology of the Pituitary, J. Mt. Sinai Hosp. **1**: 28, 1934.
6. Donaldson, H. H.: The Rat. Memoirs of the Wistar Institute of Anatomy and Biology, No. 6, Philadelphia, 1924.
7. Engle, E. T.: The Action of Anterior Pituitary and of Pregnancy Urine on the Testes of Immature Rats and Monkeys, Endocrinology **16**: 506, 1932.
8. Evans, H. M., Meyer, K., and Simpson, M. E.: The Growth and Gonad Stimulating Hormones of the Anterior Hypophysis, Memoirs of the Univ. of California, Vol. 2, 1933.
9. Hamilton, J. B.: Production of Testicular Descent in the Monkey by Male Hormone Substance, Am. J. Physiol. **119**: 325, 1937.
10. Krause, E. J.: Die Wirkung des Prolan (Aschheim-Zondek) auf die mannliche geschlechtsorgane auf Grund von Versuchen an Maus und Ratte, Klin. Wchnschr. **9**: 1493, 1930.
11. Lloyd, V. E.: Gonadotropic Hormone in the Treatment of Sterility in Man, Lancet **1**: 474, 1936.
12. Molien, M., D'Amour, F. E., and Gustavson, R. G.: Effects of Urinary Hebin Upon Immature Male Rats, Endocrinology **17**: 285, 1933.
13. Nelson, W. O.: Some Factors Involved in the Control of the Gametogenic and Endocrine Functions of the Testis. Symposia on Quantitative Biology, Cold Spring Harbor. **5**: 123, 1937.
14. Nelson, W. O., and Gallagher, T. F.: Some Effects of Androgenic Substances in the Rat, Science **84**: 230, 1936.
15. Robson, J. M., and Taylor, H.: Factors Influencing the Functional Development of the Male, Proc. Roy. Soc., London, s.B **113**: 251, 1933.
16. Rubinstein, H. S.: The Effect of the Anterior Pituitary-like Hormone on Spermatogenesis in the Human, Endocrinology **23**: 75, 1938.
17. Rubinstein, H. S.: The Production of Testicular Descent With the Water Soluble (Anterior Pituitary-like) Fraction of Pregnancy Urine, Endocrinology **18**: 475, 1934.
18. Rubinstein, H. S., and Kurland, A.: The Effect of Testosterone Propionate on Spermatogenesis in the Human, South. M. J. (In press.)
19. Smith, P. E., and Engle, E. T.: Experimental Evidence Regarding the Role of Anterior Pituitary in the Development and Regulation of the Genital System, Am. J. Anat. **40**: 159, 1927.
20. Zondek, B.: Ueber die Funktion des Ovariums, Ztschr. f. Geburtsh. u. Gynäk. **90**: 372, 1926.
21. Zondek, B.: Die Hormone des Ovariums und des Hypophysenvordenlappens, Berlin, 1931, Julius Springer.

EXCRETION OF VITAMIN C IN THE SWEAT*

IRVING S. WRIGHT, A.B., M.D., AND ELIZABETH MACLENATHEN, B.S.,
NEW YORK, N. Y.

EARLIER work, done by other workers and by ourselves, suggested that in persons who were febrile or who were subjected to temperatures sufficiently high as to cause an abnormal excretion of sweat, a substantial amount of vitamin C might be excreted through this excessive perspiration. Further studies along this line have failed to substantiate this belief.

Cornbleet, Klein, and Pace in 1936 reported that their determinations on sweat, collected from patients confined in a rubber sack during fever therapy, showed an average value of 0.55 to 0.64 mg. per cent ascorbic acid. Our figures have been considerably lower.

Sweat was collected from the face and shoulders of patients being treated in small whirlpool tanks and from the entire body of patients while under treatment in a hot circulation air cabinet at 105° F. The sweat was collected in small glass tubes and poured directly into iced tubes containing 1 c.c. of acetic acid. Five cubic centimeters aliquots were titrated with 2-6 dichlorphenolindophenol dye and the necessary corrections were made. In seventy-four determinations on 20 patients under the ordinary hospital dietary regime, the amount of ascorbic acid varied from 0.024 mg. per cent to 0.186 mg. per cent, the average being 0.041 mg. per cent. The lower figures were sometimes only very slightly higher than the water blank.

When the determinations showed the amount of ascorbic acid in the sweat to be exceedingly small, we began to question if the sweat was really an important avenue of loss of the vitamin from the body. To follow up this experiment, therefore, certain patients were given a five-hour test[2] a week prior to their fever therapy treatment, and the test was repeated again beginning five minutes prior to the start of the treatment. In this test the intravenous injection of 1,000 mg. of crystalline vitamin C was followed by a study of the urinary excretion of this substance during the succeeding five hours, according to the technique which we previously described. Sweat as well as the customary urines were collected. The five-hour urinary excretion during the treatment was lower than that of the week previous. However, as the titrations show, the diminished urinary return cannot be explained on the basis of loss through sweat. The perspiration collected over the five-hour period was divided roughly into four periods which corresponded to the rise and fall of the urinary excretion following an intravenous test

*From the Department of Medicine of the Post-Graduate Medical School and Hospital, Columbia University.

Received for publication, October 10, 1938.

The crystalline vitamin C (cebione) used in this study was supplied through the kindness of Merck and Co., Inc.

dose of vitamin C. The results obtained from these separate specimens of sweat showed slight, if any, variation from each other, or from specimens collected from the patient before his test dose, or from the range of normal values. These 52 determinations varied from 0.029 to 0.057 mg. per cent, and averaged 0.042 mg. per cent as compared with the 0.041 mg. per cent average value from persons on a normal diet. Since Gibson and Kopp have found that the gross water loss through the lungs and the skin during a five-hour period under similar conditions ranges between 1,750 and 5,500 c.c., the most vitamin C which would be lost (using our average figure) would be between 2 and 3 mg. This does not begin to compensate for the fall in the urinary excretion found when the five-hour test is run simultaneously with a fever therapy treatment. It would, therefore, appear that vitamin C is not lost in any appreciable amounts through excessive perspiration and that the lowered return in the urine is the result of increased body metabolism or some other factor.

CONCLUSIONS

1. The loss of vitamin C sustained during the spells of excessive sweating is probably best explained by an increased body metabolism and the subsequent increased utilization of ascorbic acid in the tissues.

2. The values obtained from samples of perspiration ranged from 0.024 to 0.186 mg. per cent ascorbic acid. These values did not appear to be changed significantly by the introduction of large amounts of vitamin C (1 gm. cebione) intravenously.

REFERENCES

1. Cornbleet, Theodore, Klein, R. I., and Pace, E. R.: Arch. Dermat. & Syph. **34**: 253, 1936.
2. Wright, I. S., Lilienfeld, A., and MacLenathen, E.: Arch. Int. Med. **60**: 264, 1937.
3. Gibson, J. G., and Kopp, Israel: J. Clin. Investigation **7**: 219, 1938.

THE INTRADERMAL TEST FOR VITAMIN C DETERMINATION*

IRVING S. WRIGHT, A.B., M.D., AND ELIZABETH MACLENATHEN, B.S.,
NEW YORK, N. Y.

ROTTER in 1937, and later Portnoy and Wilkinson in the earlier months of 1938, described an intradermal test by which it was claimed vitamin C subnutrition could be determined quickly. They prepared a sterile solution of 2-6 dichlorphenolindophenol containing 2 mg. of the dye in 4.9 c.c. water. An area free of hair and superficial veins was cleansed with alcohol. One-hundredth cubic centimeter of dye was then injected intradermally, forming a wheal slightly larger than 2 mm. The time of injection and of complete disappearance of the dye coloration were carefully recorded. They found that in persons of normal vitamin C nutrition the average decoloration time was less than ten minutes, while in persons with a deficiency, the time for complete decoloration was above ten minutes.

In the series which we ran according to the above procedure, we were unable to obtain results which could be correlated with the dietary histories or the blood plasma ascorbic acid levels. Our results were studied from two angles: (1) the relationship of decoloration time to the dietary history and blood values for vitamin C, and (2) the effect of the site of injection on the decoloration time. Our observations are herewith presented.

1. *Decoloration Time on the Arm in Respect to Vitamin C Intake and Blood Level.*—In this series, the dietary history and the blood determinations (done according to the macromethod of Farmer and Abt) were closely related. The intradermal tests, however, showed slight, if any, correlation.

(A) Of 11 patients with an adequate intake and normal blood values, only 3 had a decoloration time of ten minutes or less. The others varied from eleven to nineteen minutes. According to the claims of Rotter, Portnoy, and others, all of these should average below ten minutes.

(B) In the group of patients with definitely poor dietary habits and with subnormal blood levels, 4 showed decoloration times of ten minutes or less. The remaining 7 took anywhere from eleven to thirty-three minutes.

2. *Decoloration Time and Site of Injection.*—While running the intradermal tests it was noted that there was frequently a wide variation in the response to tests injected within a few centimeters of each other on the volar surface of the same arm. The difference in the time on one occasion was as great as ten minutes. The question was then raised as to whether arterial incompetence or other peripheral vascular disabilities might not play a part

*From the Department of Medicine of the Post-Graduate Medical School and Hospital, Columbia University.

Received for publication, October 10, 1938.

The crystalline vitamin C (cebione) used in this study was supplied through the kindness of Merck and Co., Inc.

in any one person's response to an intradermal test of 2-6 dichlorphenolindophenol. Skin tests for vitamin C sufficiency or depletion were, therefore, made on a group of patients in our vascular clinic. The injections were given on the volar surface of the lower arm and on the medial aspect of the calf of the leg. Where the arms were involved in a pathologic process, the tests were run on each arm and one leg. Where only the lower extremities were affected, the injections were given on each leg and one arm. Oscillometric readings were done on these patients. The following results were recorded.

(A) In one case there was a considerable discrepancy between the readings on each leg and on the right forearm.

(B) In 13 cases the readings on the different extremities agreed rather closely; i.e., within five minutes of each other.

(C) In 2 cases the decoloration times of one pair of extremities were approximately the same but differed from that on the opposite (lower or upper) extremity.

(D) In 4 cases the readings on one leg were in accord with those of the arm but differed from that of the other leg; the latter, as a rule, being longer. In this group it is interesting to note that the longer readings were frequently accompanied by diminished oscillometric reading below the knee.

All of the patients complained of an intense pain at the time of injection. This persisted for a few seconds. In several cases a hard erythematous nodule, the size of the original wheal, remained visible at the site of injection for four to five weeks.

CONCLUSIONS

We do not consider the intradermal injection of 2-6 dichlorphenolindophenol a reliable guide in the determination of the general status of the body vitamin C nutrition.

1. Variations in the decoloration time were too great to set up a normal range.

2. Readings taken at one site frequently varied considerably from those at either a close or distant site.

3. Cases of marked deficiency on two occasions fell within the normal set by Rotter, while the majority of our normals were above.

NOTE: An article by Poucher and Stubeurauch has appeared in the *J. A. M. A.* since the completion of our series. These authors likewise report the inadequacy of the intradermal test.

REFERENCES

Rotter, H.: Wien. klin. Wchnschr. **51**: 205, 1938; Nature, London **139**: 717, 1937.
Portnoy, B., and Wilkinson, J. F.: Brit. M. J. **1**: 328, 1938.
Poucher, H. G., and Stubeurauch, C. F.: J. A. M. A. **111**: 303, 1938.

POTASSIUM CYANIDE AS AN AGENT INHIBITING THE OXIDATION OF VITAMIN C IN VITRO*

IRVING S. WRIGHT, A.B., M.D., AND ELIZABETH MACLENATHEN, B.S.,
NEW YORK, N. Y.

THE report of Pijoan and Klemperer, that the addition of potassium cyanide to oxalated specimens of blood would prevent the oxidation of ascorbic acid in the blood plasma, was of great interest to us. Following their procedure (the addition of 5 mg. of potassium cyanide and 10 mg. of potassium oxalate for every 6 to 7 c.c. of blood), we ran a series of blood specimens in two batches, one with and one without the cyanide. After running over fifty such specimens, we have arrived at the conclusion that the addition of potassium cyanide does not increase the accuracy of the present method of determining the ascorbic acid values of blood plasma using the macromethod of Farmer and Abt.

All of our specimens were run immediately after the withdrawal of the blood. Where it was possible to obtain large samples, further titrations were done in six or twenty-four hours. In many cases determinations made at once were the same in both specimens. In others, the cyanide sample gave slightly higher results. When excess blood was titrated at later hours, the results were occasionally, but not uniformly, the same as in the original titration in the cyanide specimens. This was true, though not as frequent in the plain oxalated specimens, and pointed toward confirmation of the statement of Farmer and Abt that bloods differ in their loss of reduction power on standing. Where there was a drop over the six- or twenty-four-hour period, it was generally less in the cyanide specimen. This was, however, unpredictable and hence of no practical value.

CONCLUSIONS

1. The addition of potassium cyanide retards slightly, but does not prevent, the oxidation of ascorbic acid in the blood specimens. Moreover, it tends to enhance the value of the plasma as shown by recent work.[3]

2. The apparent protection against oxidation processes is so unpredictable as to be of no practical value.

REFERENCES

1. Pijoan, M., and Klemperer, F. J.: J. Clin. Investigation **16:** 443, 1937.
2. Pijoan, M., and Eddy, E. J.: J. LAB. & CLIN. MED. **22:** 1227, 1937.
3. Farmer, C. J., and Abt, A. F.: Proc. Soc. Exper. Biol. & Med. **38:** 399, 1938.
4. Friedman, G. J., Rubin, S. H., and Kias, W.: Proc. Soc. Exper. Biol. & Med. **38:** 358, 1938.

*From the Department of Medicine of the Post-Graduate Medical School and Hospital, Columbia University.

Received for publication, October 10, 1938.

BIOCHEMICAL OBSERVATIONS IN HYPOGLYCEMIA INDUCED BY INSULIN*

II.† Behavior of Blood Gases in Relation to the Hypoglycemic State

Edward F. Rosenberg, M.D., Rochester, Minn.

RECENTLY while treating patients with schizophrenia by means of insulin, I made a number of biochemical observations during the hypoglycemic periods. Observations of the effect of insulin on the level of the blood uric acid have already been reported.[8] Among my data are included a group of studies of the behavior of the gases of the blood during the hypoglycemic state. Some scattered reports on this subject are available in the literature, but they are contradictory and are not extensive. I am submitting these studies because there appears to be a need for more observations of these effects.

METHOD

Venous blood was drawn from an elbow vein without aid of tourniquet and avoiding stasis. The specimen was collected in a sterile syringe containing about 5 c.c. of sterile mineral oil which was free of air bubbles. The blood was immediately transferred to a small glass beaker containing a few crystals of potassium oxalate covered by a layer of sterile mineral oil. The blood was stirred immediately and rather gently with a small glass rod. These samples were analyzed‡ for oxygen and carbon dioxide content within thirty minutes after the withdrawal of the samples. For determination of the oxygen capacity the samples were equilibrated with room air and at room temperature by rotating in a separatory funnel until maximal exposure of the blood was certain. This required fifteen minutes or longer. The technique and apparatus used to determine the oxygen and carbon dioxide of blood samples were those described by Van Slyke and Neill. All determinations were made in duplicate simultaneously on two machines. Oxygen saturation of venous blood is determined by dividing the value for oxygen content by the oxygen capacity. These studies were conducted with schizophrenic patients who were otherwise "metabolically normal."

OBSERVATIONS

If one administers a large dose of insulin and allows hypoglycemia to develop, the level of oxygen content of venous blood increases markedly. The saturation with oxygen under these circumstances frequently reaches to a

*From the Mayo Foundation, Rochester.

†The report of the first study in this series was not designated by a Roman numeral. Its title appears in the list of references appended to this report.

Abridgment of thesis submitted to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of M.S. in Medicine.

Received for publication, October 20, 1938.

‡Analysis made by Miss Elizabeth Maclay in the laboratory of Dr. A. E. Osterberg.

TABLE I

GASES IN VENOUS BLOOD IN RELATION TO HYPOGLYCEMIA INDUCED BY INSULIN

DATE	CASE NUMBER	UNITS OF INSULIN AT 7:20 A.M.	TIME	CARBON DIOXIDE CONTENT	OXYGEN CONTENT	OXYGEN SATURATION	OXYGEN CAPACITY	BLOOD SUGAR*	COMMENT
				Volumes per cent	*Volumes per cent*	*Volumes per cent*	*Volumes per cent*	*Mg. per 100 c.c.*	
3/17/37	1	60	7:25 A.M.	52.9	13.1	67.9	19.3	102.0	
			10:09 A.M.	55.6	16.2	84.6	Not done	22.0	In hypoglycemic coma
3/30/37	2	140	7:20 A.M.	57.6	11.7	61.5	19.1	117.6	
			10:00 A.M.	52.2	21.2	92.6	22.9	47.1	Marked hypoglycemic symptoms
3/30/37	3	90	7:20 A.M.	61.9	10.5	54.3	19.3	138.9	
			10:00 A.M.	57.2	17.3	86.5	20.0	58.8	Marked hypoglycemic symptoms
4/12/37	2	160	7:29 A.M.		10.7	54.2	19.88	94.3	
			10:30 A.M.		19.8	87.7	22.58	31.4	In hypoglycemic coma
			11:30 A.M.	150 gm. sucrose through nasal tube					
			1:30 P.M.		5.54	23.95	23.14	100	Conscious—appears normal

*Micromethod of Folin

point equaling that of arterial blood. Also, during hypoglycemia, the capacity of venous blood to carry oxygen is regularly somewhat increased. The response of the carbon dioxide carrying capacity to hypoglycemia is not always the same. In some studies slight rises were detected, whereas in other instances the effect of hypoglycemia was to induce a slight fall (Table I).

In each of the four studies the appearance of hypoglycemia was accompanied by a substantial rise in the oxygen saturation and oxygen content of the venous blood. In Cases 2 and 3 considerable increases in the values for oxygen capacity also appeared. In study 4 (Case 2), observations made two hours after the termination of the hypoglycemic episode showed that the oxygen content and oxygen saturation had returned to normal values for venous blood. At this time the color of the venous blood had again become dark.

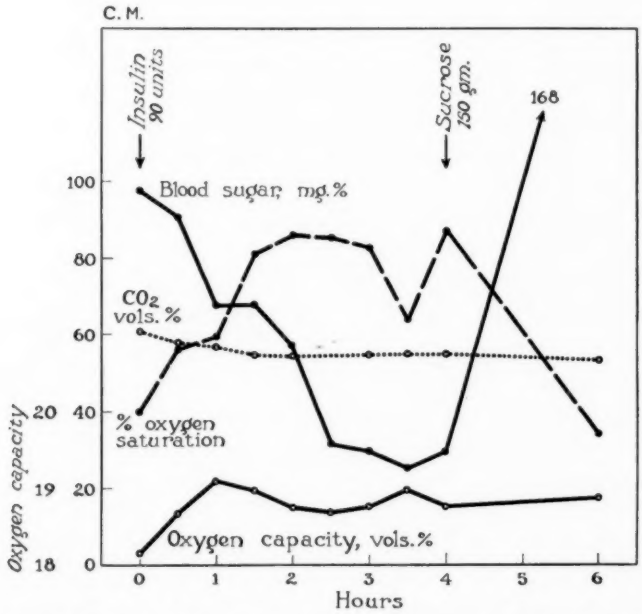


Fig. 1.—Gases in venous blood in relation to hypoglycemia induced by insulin. Development of the hypoglycemic state is accompanied by a marked rise in oxygen saturation, a rise in oxygen capacity, and a slight lowering of the level of carbon dioxide content.

The study on patient 3 illustrates the rates of change in the gas content of blood during hypoglycemia and establishes in the form of curves the speed of appearance and disappearance of these effects (Table II, Fig. 1).

In this study ninety minutes after the injection of a large dose of insulin the oxygen content of venous blood had more than doubled, and this increase was maintained more or less constantly throughout the period of hypoglycemia. Carbohydrate was fed four hours after beginning the study. At the sixth hour when the patient was completely recovered from hypoglycemia, the value for oxygen content and oxygen saturation had returned to normal. A definite rise in the oxygen-carrying capacity of blood was likewise present one hour after the insulin injection. Not only was this increase maintained throughout the hypoglycemic period; it persisted and was present two hours

TABLE II

GASES IN VENOUS BLOOD REPRESENTED IN FIG. 1

TIME AT WHICH BLOOD SAMPLE WAS TAKEN	CARBON DIOXIDE CONTENT	OXYGEN CONTENT	OXYGEN SATURATION	OXYGEN CAPACITY	BLOOD SUGAR*
	Volumes per cent	*Volumes per cent*	*Per cent*	*Volumes per cent*	*Mg. per 100 c.c.*
April 22, 1937. Patient 3; Insulin 90 Units at 7:15 A.M.					
7:15 A.M.	60.2	7.25	40.0	18.14	97.6
7:45 A.M.	58.0	9.40	56.3	18.64	90.9
8:15 A.M.	56.9	11.30	59.4	19.08	67.8
8:45 A.M.	54.9	15.20	81.2	18.96	67.8
9:15 A.M.	54.1	16.10	85.9	18.73	57.1
9:45 A.M.	54.4	16.00	85.7	18.67	31.2
10:15 A.M.	54.7	15.50	82.7	18.75	29.4
10:45 A.M.	54.9	12.30	64.8	18.98	25.0
11:15 A.M.	54.9	16.3	86.9	18.76	29.4
11:17 A.M.	150 gm. sucrose through nasal tube				
1:15 P.M.	53.2	8.45	44.8	18.87	168.0

*Micromethod of Folin.

after administration of a large amount of carbohydrate had restored the blood sugar to normal and the patient to consciousness. Thus not all effects of insulin are counteracted promptly by carbohydrate feeding. The charted curve of the values for the carbon dioxide content of the blood shows a fall during the first two hours following the administration of insulin. This curve then reaches a level which is maintained throughout the remainder of the observation period. The regular form of this charted curve suggests that the change is not due to chance, but that it is the result of some definite change in blood chemistry as yet not understood.

Control Observations.—Two studies were made on patients of the same group under similar circumstances. The patients were in bed; one was allowed

TABLE III

GASES IN VENOUS BLOOD: CONTROL OBSERVATIONS—NO INSULIN GIVEN

DATE	CASE NUMBER	TIME	CARBON DIOXIDE CONTENT	OXYGEN CONTENT	OXYGEN SATURATION	OXYGEN CAPACITY	BLOOD SUGAR*	COMMENT
			Volumes per cent	*Volumes per cent*	*Volumes per cent*	*Volumes per cent*	*Milligrams per 100 c.c.*	
4/14/37	4	7:35 A.M.	56.8	7.1	36.1	19.4	117.6	Breakfast allowed at 7 A.M.
		10:00 A.M.	58.2	9.2	49.8	18.4	116.3	
		11:30 A.M.	150 gm. sucrose in 500 c.c. milk by mouth					
		1:30 P.M.	61.5	5.7	30.7	18.8	125	
4/15/37	3	7:00 A.M.	56.2	10.4	53.7	19.4	133.3	No breakfast allowed
		10:30 A.M.	55.0	13.7	67.0	20.5	124.3	
		11:30 A.M.	150 gm. sucrose in 500 c.c. milk by mouth					
		1:30 P.M.	55.3	14.0	70.9	19.7	127.0	

*Micromethod of Folin.

breakfast and the other was deprived of food. One hundred and fifty grams of sucrose were administered to each by mouth at a time corresponding to the carbohydrate feedings in the above studies (Table III).

Contrasts between the findings in these control studies and those studies in which insulin was administered and hypoglycemia allowed to appear are obvious. No significant changes appeared in the oxygen capacity and the degree of saturation of the venous blood with oxygen remained within normal limits for venous blood in all observations on control subjects. The margin of error of the determination of oxygen capacity is usually taken to be 0.5 volume per cent. For this reason, the changes in oxygen capacity in the control study are thought to be insignificant, whereas the degree of change in oxygen capacity noted in the studies during hypoglycemia are thought to be significant.

COMMENT

In 1923 Olmsted and Logan studied the effect of insulin on the central nervous system and noted that arterial blood of cats was very dark and venous in color when the blood sugar was low. They stated that the approach of the blood sugar to the convulsive level could be foretold by observing the color and character of the blood. They also described "many points of resemblance between an insulin convulsion and an asphyxial convulsion such as that caused by clamping the trachea of a cat or rabbit." Later Olmsted and Taylor reported that the saturation of hemoglobin with oxygen remains constant for three hours after administration of a convulsive dose of insulin, that is, until one hour before a convulsion; then it falls. In one of their experiments at the beginning of a convulsion the oxyhemoglobin fell to 75 per cent from an original level of 88 per cent, and after the convulsions it returned to nearly normal. Klein and Holzer found that in hypoglycemia the alkali reserve, as measured by changes in the carbon dioxide combining power, did not vary beyond the limits of normal, but patients in whom the more violent symptoms appeared, such as sweating, palpitation, shivering, hunger, thirst, and so forth, did show a slight lowering of the alkali reserve. They also noted that in contrast to the observations of Olmsted and Logan in rabbits, the red color of venous blood almost equals that of arterial blood during hypoglycemia in man. Later they[3] reported studies of the oxygen saturation of venous blood during hypoglycemia, using the Barcroft apparatus. They found that the oxygen saturation of venous blood in thirteen instances of hypoglycemia varied from 86 per cent to 97 per cent. Wiechmann and Koch also observed this color change, as did Lauter and Baumann, who noted also a slight increase in the oxygen capacity of the venous blood during hypoglycemia. Briggs, Koechig, Doisy, and Weber have stated that during hypoglycemia there occurs a rise in the value of lactic acid in the blood. This may account for the fall in carbon dioxide capacity which I observed in some of my studies.

The cause of the increased oxygen saturation of venous blood in hypoglycemia is not clear. One probable explanation is suggested by the observation of Ernstene and Altschule of an average of 29 per cent increase in cir-

culating blood volume per minute during hypoglycemia. If no alteration in oxygen utilization in the tissues occurs in hypoglycemic states, then with a greater circulating volume per minute less oxygen would be removed from a unit volume of blood and the venous blood could be expected to be more saturated with oxygen.

The increased carrying capacity of blood for oxygen during hypoglycemia is probably due to hemoconcentration. Further observations, including studies of behavior of the values for hemoglobin, hematocrit, erythrocyte count, plasma chlorides, and serum proteins, support this belief and will be reported later.

CONCLUSIONS

1. The development of the hypoglycemic state is associated with a marked increase in the concentration of oxygen in the venous blood. This concentration reaches values usually found in studies of the arterial blood.

2. The blood has a greater oxygen-carrying capacity during hypoglycemia than normally.

3. In one instance a slight but steady fall occurred in the value for carbon dioxide content of venous blood.

REFERENCES

1. Briggs, A. P., Koechig, Irene, Doisy, E. A., and Weber, C. J.: Some Changes in the Composition of Blood Due to the Injection of Insulin, J. Biol. Chem. **58**: 721, 1924.
2. Ernstene, A. C., and Altschule, M. D.: The Effect of Insulin Hypoglycemia on the Circulation, J. Clin. Investigation **10**: 521, 1931.
3. Holzer, H., and Klein, O.: Ueber die Sauerstoffsättigung des Venenblutes peripherer Gefässgebiete im Insulinschock, München. med. Wchnschr. **2**: 1284, 1928.
4. Klein, O., and Holzer, H.: Weitere Mitteilungen über Insulinhypoglykämie und Insulinschock beim Menschen, Ztschr. f. klin. Med. **107**: 94, 1928.
5. Lauter, S., and Baumann, H.: Kreislauf und Atmung im hypoglykämischen Zustand, Deutsches Arch. f. klin. Med. **163**: 161, 1929.
6. Olmsted, J. M. D., and Logan, H. D.: The Effect of Insulin on the Central Nervous System and Its Relation to the Pituitary Body, Am. J. Physiol. **66**: 437, 1923.
7. Olmsted, J. M. D., and Taylor, A. C.: The Effect of Insulin on the Oxygen Saturation of Hemoglobin, J. Biol. Chem. (Scientific Proceedings) **59**: 30, 1934.
8. Rosenberg, E. F.: Effect of Insulin on the Concentration of Uric Acid in the Blood, J. Clin. Investigation **17**: 233, 1938.
9. Van Slyke, D. D., and Neill, J. M.: The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, J. Biol. Chem. **61**: 523, 1924.
10. Wiechmann, Ernst, and Koch, Fritz: Ueber die Sauerstoffsättigung des Venenblutes peripherer Gefässgebiete im Insulinschock, München. med. Wchnschr. **2**: 1800, 1928.
11. Wiechmann, Ernst, and Koch, Fritz: Untersuchungen über den hypoglykämischen Zustand nach Insulininjektion. III. Mitteilung. Das Verhalten des Kreislaufs im hypoglykämischen Zustand, Deutsches Arch. f. klin. Med. **163**: 176, 1929.

BIOCHEMICAL OBSERVATIONS IN HYPOGLYCEMIA INDUCED BY INSULIN*

III. Glycorrhachia and Hypoglycemia†

Edward F. Rosenberg, M.D., Rochester, Minn.

MANY outstanding clinical phenomena of the hypoglycemic state are manifestly due to disturbances within the central nervous system.[5] These symptoms presumably reflect alterations in the chemistry of the nerve tissues. Studies of the behavior of constituents of the cerebrospinal fluid might be expected to throw some light on the nature of the changes which occur in the brain, yet so far as I am aware, only one paper dealing with experimental effects of insulin hypoglycemia on the cerebrospinal fluid of man and only a few reports of the results of animal experiments have been published. Accordingly, I am submitting the following data which bear on this subject.

METHOD

Lumbar puncture and venipuncture were performed on fasting patients at about 7 A.M. and samples were removed for analyses. Following this a "coma dose" of insulin was injected. Thereafter until hypoglycemia was terminated by administration of sucrose, lumbar puncture and venipuncture were repeated hourly. Determinations were also made during the afternoons following termination of coma, until the sugar values of the cerebrospinal fluid had returned to approximately their original level. All patients used in these studies were schizophrenics in whom, except for functional mental disturbance, there was no evidence of disease.

OBSERVATIONS

If one administers a large amount of insulin and induces a profound fall in the level of the blood sugar, the cerebrospinal fluid sugar level also falls, though not so rapidly as does the blood sugar (Table I, Fig. 1).

For the purposes of a control I made the following observations. A patient was kept at rest in bed. No food and no insulin were given in the morning (Table II). Thus in a fasting patient to whom no insulin is administered, the sugar content of the cerebrospinal fluid varies somewhat as does the blood sugar during the course of the day. These variations are small and have no definite pattern.

The level of chlorides in the cerebrospinal fluid in the second study (Table I) was not influenced by the effects of the insulin. In the third study (Table I) the afternoon chloride levels were somewhat lower than those made

*From the Mayo Foundation, Rochester.

†Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of M.S. in Medicine.

Received for publication, October 20, 1938.

TABLE I

STUDIES OF CEREBROSPINAL FLUID IN RELATION TO HYPOGLYCEMIA

DATE	INSULIN UNITS GIVEN AT 7 A.M.	CASE NUMBER	TIME	BLOOD SUGAR*	CEREBROSPINAL FLUID							COMMENT
					SUGAR*	CELLS	NONNE	PROTEIN	CHLORIDES‡	WATER PRESSURE		
										BEFORE	AFTER	
				Mg. per 100 c.c.	*Mg. per 100 c.c.*			*Mg. per 100 c.c.*†		*Cm.*	*Cm.*	
1/28/37	70	1	7 A.M.	112	79.5							
			8 A.M.	77.6	75.5							
			9 A.M.	25.6	67.5							In coma
			10 A.M.	21.6	65.6							In coma
			11 A.M.	25.1	42.5							In coma
			11:05 A.M.	150 gm. sucrose in 500 c.c. water through nasal tube								
			1 P.M.	148	46.3							Awake
			3 P.M.	127.5	65.6							Awake
			5 P.M.	130.0	74.5							Awake
2/27/37	70	1	7 A.M.	97.1	80	4 Lymphs.§ Few R.B.C.	+	45	726	15	8	
			8 A.M.	86.0	75.2	5 Lymphs. Few R.B.C.	+	50	726	8	6	
			9 A.M.	31.2	65.8	Bloody	+	100	720	7	6	In coma
			10 A.M.	29.0	38.1	5 Lymphs. Few R.B.C.	+	50	722	8	6	In coma
			11 A.M.	20.7	31.4	4 Lymphs. Few R.B.C.	+	60	722	8	6	In coma
			11:05 A.M.	150 gm. sucrose in 500 c.c. water through nasal tube								
			1 P.M.	137	32.2	10 Lymphs. Few R.B.C.	+	60	722	8	6	Awake
			3 P.M.	119.7	80.9	15 Lymphs. Few R.B.C.	+	60	726	8	6	Awake
			5 P.M.	80	54			Not done		10	9	Awake

TABLE I—CONT'D

2/ 5/37	70	2	7 A.M.	108.1	70				720			
			8 A.M.	90.1	41.3				726			
			9 A.M.	26.0	33				720			In coma
			10 A.M.	17.7	45				726			In coma
			11 A.M.	19.2	32				723			In coma
			11:05 A.M.	150 gm. sucrose in 500 c.c. water through nasal tube								
			1 P.M.	186.9	47.6				660			Awake
			3 P.M.	156.2	51.3				687			Awake
			5 P.M.	133.3	83.3				Not done			Awake
5/ 5/37	100	3	7:45 A.M.	95	68							
			8:45 A.M.	73	58							
			9:45 A.M.	57	34							Severe hypo-glycemic symptoms
			10:45 A.M.	23	25							In coma
			12:30 P.M.	7	23							In coma
			12:35 P.M.	150 gm. sucrose in 500 c.c. water through nasal tube								
			1:45 P.M.	129	40.5							Awake
			3:45 P.M.	141	54.0							Awake

*Micromethod of Folin.
†Kingsbury, Clark, Williams, and Post method.[8]
‡Osterberg-Schmidt modification of Whiteborn method.[6]
§Lymphs. = lymphocytes; R.B.C. = erythrocytes.

during the hypoglycemic period. These differences probably resulted from the fact that patient 2 sweated profusely during hypoglycemia and doubtless lost chlorides in perspiration, whereas patient 1 remained dry throughout the studies.

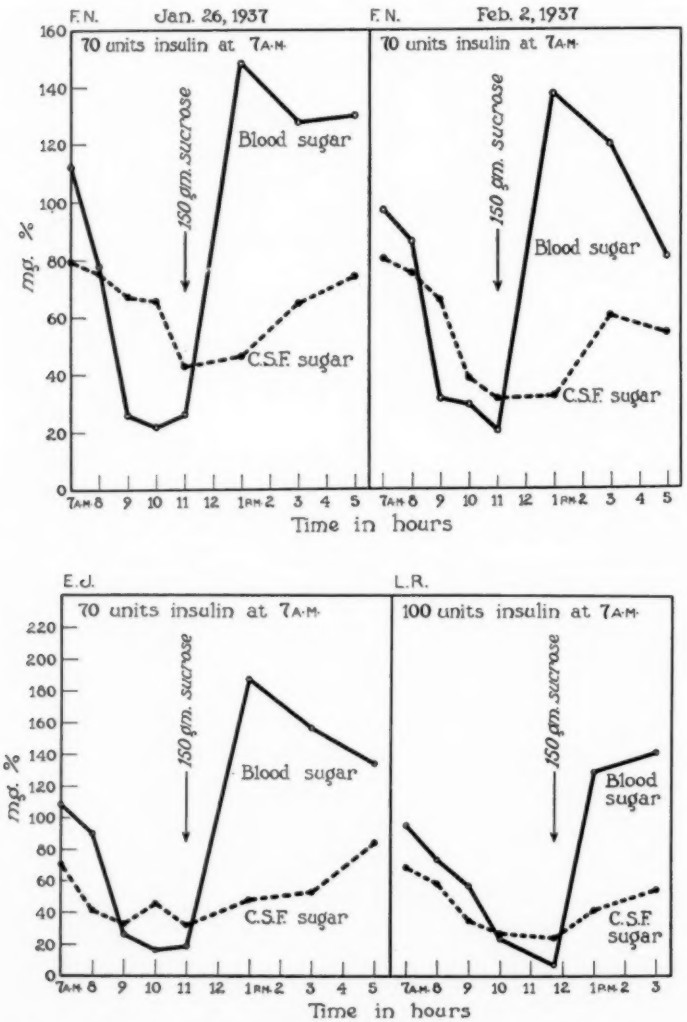


Fig. 1.—Studies illustrating the relationship between the level of *d*-glucose in the blood and in cerebrospinal fluid during hypoglycemia induced by insulin.

In the second study (Table I) there does not appear to be any significant alteration in the level of protein in the cerebrospinal fluid as a result of insulin administration and no pressure changes attributable to the action of insulin. The erythrocytes probably resulted from trauma.

COMMENT

Rathery and Sigwald observed the effect of insulin hypoglycemia on the sugar content of the cerebrospinal fluid of dogs. They stated that in hypo-

glycemia the level of sugar in the cerebrospinal fluid dropped but not so far relatively as did the blood sugar. In one instance blood sugar fell from 120 mg. per 100 c.c. to 37 mg. per 100 c.c., and the cerebrospinal fluid sugar fell from 80 mg. per 100 c.c. to 54 mg. per 100 c.c. In their second instance blood sugar fell from 86 mg. per 100 c.c. to 26 mg. per 100 c.c., the cerebrospinal fluid sugar falling from 87 mg. per 100 c.c. to 43 mg. per 100 c.c. As to the pressure of the cerebrospinal fluid in hypoglycemia they always found a relative hypotension during the hypoglycemic period as compared with the original pressure.

TABLE II

CONTROL STUDY OF CEREBROSPINAL FLUID IN RELATION TO BLOOD SUGAR

TIME SAMPLES WERE TAKEN	BLOOD SUGAR*	CEREBROSPINAL FLUID SUGAR*
	Mg. per 100 c.c.	*Mg. per 100 c.c.*
7 A.M.	95.6	57
9:30 A.M.	88	62
11:30 A.M.	95	57
12 Noon	"lunch"	
1:45 P.M.	113	65
3:45 P.M.	122	68

*Micromethod of Folin.

Cahane stated that there is a close relationship between the quantity of sugar in blood and in the cerebrospinal fluid. Lowering of the level of the blood sugar appeared half an hour after administration of insulin. One hour after the injection the level of sugar in the cerebrospinal fluid was lowered. The fall in level of the cerebrospinal fluid sugar was relatively less than that of the blood sugar. These observations were made on 4 human beings and 2 dogs; each subject was studied after administration of 20 units of insulin. No observations of the recovery phase were reported.

Kasahara and Uetani have reported studies of the cerebrospinal fluid sugar in the rabbit following insulin administration. Insulin doses of 0.5 unit per kilogram induced hypoglycorrhachia in the rabbit which attained its maximum between the second and third hours after the injection. The level of the cerebrospinal fluid sugar usually had returned to normal after seven hours.

Numerous individual observations of the level of the cerebrospinal fluid sugar of patients in hypoglycemia have undoubtedly been studied and some have been reported. Davis and Brown have published such a study. They observed a patient in hypoglycemic coma and administered 100 gm. of *d*-glucose intravenously, but the patient did not respond. Blood sugar after death was 176 mg. per 100 c.c. but the spinal fluid sugar was only 25 mg. per 100 c.c. Then they made some studies in dogs. First they recorded the blood and cerebrospinal fluid sugar values and then administered insulin. When the dogs were in hypoglycemia, these values were redetermined. Insulin caused the usual fall in blood sugar and the cerebrospinal fluid sugar fell also. The drop in blood sugar was more marked than the drop in sugar level of the cerebrospinal fluid.

Drabkin and Ravdin found a rise in the pressure of the cerebrospinal fluid of hydrated dogs during hypoglycemia, but no rise in the pressure of the

cerebrospinal fluid in dehydrated dogs under the same circumstances. Patient 1 in my second study (Table I) did not have any perceptible rise during hypoglycemia in the pressure of the cerebrospinal fluid as measured by the water manometer.

CONCLUSIONS

1. Lowering of the level of blood sugar following the administration of insulin is accompanied in man by a corresponding but slower fall in level of cerebrospinal fluid sugar.

2. Administration of carbohydrate to a patient in hypoglycemic coma induces a rapid rise in blood sugar level and a corresponding but slower rise in level of cerebrospinal fluid sugar.

3. Administration of insulin has no definite influence on the level of chlorides or protein in cerebrospinal fluid.

4. No changes in pressure of cerebrospinal fluid which can be attributed to the effect of insulin occurred during insulin hypoglycemia among these patients.

REFERENCES

1. Cahane, M.: Influence de l'insuline sur la glycorachie, Compt. rend. Soc. de biol. **111**: 111, 1932.
2. Davis, Reuben, and Brown, Herman: Spinal Fluid Sugar Determinations in Experimental Hypoglycemia of Dogs, J. LAB. & CLIN. MED. **19**: 1049, 1934.
3. Drabkin, D. L., and Ravdin, I. S.: The Mechanism of Convulsions in Insulin Hypoglycemia. Interrelationship of Blood Concentration, Cerebrospinal Pressure and Convulsions, Am. J. Physiol. **118**: 174, 1937.
4. Kasahara, Michio, and Uetani, Nikichi: The Effect of Insulin Upon the Reducing Substance in the Cerebrospinal Fluid of Normal Rabbits, J. Biol. Chem. **59**: 433, 1924.
5. Moersch, F. P., and Kernohan, J. W.: Hypoglycemia; Neurologic and Neuropathologic Studies, Arch. Neurol. & Psychiat. **39**: 242, 1938.
6. Osterberg, A. E., and Schmidt, Edna V.: The Estimation of Plasma Chlorides, J. LAB. & CLIN. MED. **13**: 172, 1927.
7. Rathery, F., and Sigwald, J.: Etude expérimentale des modifications humorales dans l'hypoglycémie insulinique chez le chien, Compt. rend. Soc. de biol. **107**: 1074, 1931.
8. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods, ed. 8, Philadelphia, 1935, W. B. Saunders Co., p. 544.

PLATELET STUDIES IN NORMAL MEN AND WOMEN (MENSTRUATING AND NONMENSTRUATING) AND SUBJECTS WITH BLEEDING DISORDERS*

Counts, Disintegration Rates, and Intradermal Platelet Injections

Pearl Lee, M.D., and Betty Nims Erickson, M.A., Detroit, Mich.

THE role played by the platelets in the mechanism of blood clotting has long furnished investigators with an intriguing problem. Extensive research over many years has yielded important facts concerning their origin, morphology, and physiology. More recently, accurate knowledge of their chemical composition has been added.[1] Nevertheless, the exact manner in which they enter into the normal clotting process, and the cause of their variations in the bleeding disorders is still very imperfectly understood.

The lack of adequate methods has, in most instances, limited the clinical study of platelets to an enumeration of the total count. Recently, Olef[2] has evolved a method for making total counts which provides for a differential on the basis of size. Probably the best method available for determining the relative fragility of the platelets in health and disease[3-7] has also been developed by Olef.[8]† He styles his determination the "Disintegration Rate." The greatest advantage of this method is that it makes possible a comparison of the differential formula of the initial count with that obtained after the disintegration period.

Using these improved methods of approach to platelet behavior, a study was made of total and differential platelet counts, and disintegration rates accompanied by platelet lipid determinations.[9] Normal men and women, menstruating women, hemophiliacs, and patients with thrombopenic purpuras were subjects of study. The results of this study are supplemented in this paper by the results of several years' work on skin sensitivity to washed platelet suspensions. The skin sensitivity is correlated with the total platelet count and disintegration rate.

Olef's method[2] for total and differential platelet counting is time-consuming and requires considerable experience in its use for the production of successful results. The method is an indirect one, involving the use of a solution of sodium metaphosphate to prevent clumping and disintegration. A drop of the metaphosphate is placed over the site of the finger puncture so that the blood is mixed directly with the diluting fluid and does not contact the skin.

*From the Children's Hospital of Michigan and the Research Laboratory, Children's Fund of Michigan.

Received for publication, October 21, 1938.

†Olef's technique for disintegration rate[8] was modified slightly by placing the paraffin cup containing the blood in the incubator at 37° C. for six hours, instead of the refrigerator for eight hours.

TABLE I

TOTAL AND DIFFERENTIAL PLATELET COUNTS*

(All platelet counts expressed in thousands)

SUBJECTS			NUMBER OF COUNTS	TOTAL PLATELET RANGE	COUNT† NEAREST MEAN	COUNT NEAREST MEAN DIFFERENTIAL COUNT					
NO.	AGE	SEX				GROUP I		GROUP II		GROUP III	
							per cent		*per cent*		*per cent*
14 Normal Men and Nonmenstruating Women											
1	31	M	3	788-870	800	56	7	648	81	96	12
1	31	M	3	369-458	427	192	45	222	52	13	3‡
2	22	M	5	850-1030	957	57	6	766	80	134	14
2	22	M	6	520-793	626	319	51	301	48	6	1
3	32	M	6	480-690	570	188	33	342	60	40	7
3	32	M	2	490-570	490	245	50	240	49	5	1
4	27	M	4	477-570	508	137	27	371	73	0	
5	23	F	2	440-693	440	114	26	326	74	0	
6	31	F	5	328-712	424	84	20	280	66	60	14
6	31	F	4	542-813	715	350	49	365	51	0	
6	31	F	6	302-421	371	152	41	219	59	0	**
7	27	F	3	726-928	811	219	27	560	69	32	4
8	30	F	2	511-586	511	128	25	383	75	0	
9	42	F	3	629-882	828	248	30	580	70	0	
9	42	F	4	356-407	378	159	42	215	57	4	1§
9	42	F	2	423-448	423	288	68	135	32	0	
10	25	F	2	574-613	574	86	15	465	81	23	4
11	25	F	4	578-801	693	249	36	367	53	76	11
12	24	F	4	585-873	792	340	43	428	54	24	3
13	31	F	3	1026-1206	1098	318	29	758	69	22	2
14	27	F	3	639-761	711	242	34	455	64	14	2
Total counts			76								
5 Normal Women, Menstruating											
5	23	F	4	400-539	428	111	26	287	67	30	7
5	23	F	2	539-621	539	140	26	377	70	22	4
6	31	F	3	481-502	485	218	45	267	55	0	
7	27	F	4	387-582	461	157	34	300	46	4	1
8	30	F	3	426-471	451	221	49	230	51	0	
9	42	F	2	504-558	504	222	44	257	51	25	5
9	42	F	2	330-688	330	122	37	208	63	0	
Total counts			20								

*All counts in each group were taken within thirty days. Thirty days or more elapsed between groups of counts on the same individual.

†When only two counts were taken, the lowest total was used as count nearest mean.

‡Acute cold and sinusitis for ten days.

§Sinus infection and severe cough. Five days elapsed between this and the preceding period.

**Acute cold and sinusitis.

After transferring the mixture to a paraffin cup, fresh preparations are made for counting. A simultaneous erythrocyte count is also necessary, to calculate the absolute number of platelets. The platelets are differentiated into three classes according to size, using the red blood cell as a standard. Group I is one-fourth the size of the red blood cell, Group II is one-third, and Group III one-half. This method gives higher total counts than most of those used, mainly because there is less platelet destruction involved in the preparation of the slides. Further details of this method may be found in the author's paper.[2]

All blood samples were taken between 10 and 11 o'clock in the morning, approximately two hours after eating. To establish a normal total figure for

any individual, counts were made on three successive days. A variation of 100,000 was allowed as the limits of error.

Observations were made on 4 normal men, 10 normal women, when not menstruating, and 9 hemophilic children. Five of the women were studied during menstruation. The normal individuals were all in good health and had no history of unusual bleeding. The hemophiliacs had been under observation for several years. Their histories, and laboratory and clinical findings were typical of this bleeding disorder.

Table I presents the normal total and differential studies. It consists of the counts divided into 21 groups, made on 14 different subjects. The count nearest the mean for the group is given with its differential. This method of selection is followed in all the tables. When only two counts were taken, the lowest is used with its differential.

Inspection of the total counts (Table I) reveals that the groups for the same individual vary widely. This bears out Brakefield's report[11] of wide variations from week to week upon the same subject. The counts in any group, with one exception, were taken within intervals of thirty days. Where there was a marked drop, it was usually associated with an acute cold and sinusitis. Subjects 1, 6, and 9 demonstrate these findings. There are no differences apparent between the men and women. Seven groups of counts presented in Table I were made upon 5 of the normal women when they were menstruating.

TABLE II

TOTAL PLATELET AND DIFFERENTIAL COUNTS

HEMOPHILIACS

(All platelet counts expressed in thousands)

MALE SUBJECTS		NUMBER OF COUNTS	TOTAL PLATELET RANGE	COUNT NEAREST MEAN*	COUNT NEAREST MEAN DIFFERENTIAL COUNT					
NO.	AGE				GROUP I		GROUP II		GROUP III	
						per cent		*per cent*		*per cent*
15	13	3	450-1188	748	524	70	210	28	14	2
16	7	2	546-640	546	202	37	344	63	0	–
17	8	2	252-506	252	126	50	101	40	25	10
18	4	4	991-1157	1013	597	59	416	41	0	–
19	9	2	944-974	944	604	65	331	35	9	1
20	13	1	450	450	270	60	166	37	14	3
21	15	1	414	414	166	40	248	60	0	–
22	6	2	317-1441	317	101	32	216	68	0	–
23	8	1	639	639	256	40	377	59	6	1
Total counts		18								

*When only two counts were taken, the lowest was used.

The same type of information is given for hemophiliacs in Table II. Here a total of 18 counts is divided into nine groups. Nine different individuals are the subjects of the study.

A comparative analysis of Tables I and II may be made by comparing the group averages and ranges for the counts nearest the mean according to the frequency of the occurrence of the total counts at three levels (400-600 thousand, 600-800 thousand, and over 800 thousand). These figures are given in Table III.

It may be seen that about 63 per cent of the normal total counts are included at the lowest level. All the seven groups made on menstruating women are in the 400-600 thousand range. In this small series, the counts during menstruation are definitely lower than in the group including both men and nonmenstruating women. Genell[12] has pointed out similar decreases in the counts during menstruation.

TABLE III

AVERAGES AND RANGES OF DIFFERENTIAL PLATELET COUNTS OF TOTAL COUNTS AT THREE RANGE LEVELS

	NUMBER OF SUBJECTS	GROUPS OF DETERMINATIONS	FREQUENCY DISTRIBUTION ACCORDING TO TOTAL COUNT LEVELS		
			400-600 THOUSAND	600-800 THOUSAND	800 + THOUSAND
Normal M & F (Nonmenstruating)	14 (4M, 10F)	21	11	5	5
Group I:					
Average			161	300	180
Range			84-288	242-350	56-318
Group II:					
Average			291	383	662
Range			135-465	301-455	560-766
Normal F (Nonmenstruating)*	5	9	6	1	2
Group I:					
Average			154	350	234
Range			84-288		219-248
Group II:					
Average			260	365	570
Range			135-383		560-580
Normal F (Menstruating)*	5	7	7	0	0
Group I:					
Average			170		
Range			111-222		
Group II:					
Average			275		
Range			208-377		
Hemophiliacs† (Children)	9	9	5	2	2
Group I:					
Average			173	390	600
Range			101-270	256-524	597-604
Group II:					
Average			215	294	374
Range			101-344	210-377	331-416

*Same 5 women.

†Two counts in this group are below 350,000.

No significant difference between the different types of subjects is found in the distribution of Groups I and II, in the lowest total count level. However, there is a definite difference in the highest level counts (800,000 or over) for the normals and the hemophiliacs. In the high counts for the normals a much greater percentage of Group II is present than in the 400-600 thousand class. The hemophiliacs reverse this picture, i.e., they show a marked increase in the percentage of Group I in the high counts.

Olef[13] states in his paper on the clinical significance of the differential platelet count that in the study of 45 adults he obtained the following average: Group I, 18.6 per cent; Group II, 63.3 per cent; Group III, 17.4 per cent. The method as used here gave averages of 33 per cent for Group I, and 63 per cent for Group II. Group III was usually so small that it has been ignored in the general averages.

Olef included a Group IV, consisting of odd-shaped platelets, in the differential. The number of these is so small that we have ignored his group completely.

DISINTEGRATION RATES

In Table IV the disintegration rates for normal women are presented. The subjects when not menstruating show a decrease in total count after six hours, ranging from 30 per cent to 52 per cent, with an average of 41. The women when menstruating have disintegration rates varying from –16 to +39, with an average of +2. (The "+" sign indicates that the total count was increased by that per cent.)

Table V presents 15 disintegration rates performed on 9 different hemophiliacs. The rates ranged from –27 to +89, with an average of +2.5. An analysis of the changes in the differential at the end of the six-hour period is given in Table VI, based upon the results contained in Tables IV and V.

A comparison of the disintegration rate for the various subjects shows that the rate is much faster for the normal nonmenstruating women than for any of the other groups presented. The range was from –30 per cent to –52 per cent, with an average of –41 per cent. In this paper we have considered anything slower than –30 to be retarded. Olef[8] regarded any figure faster than –10 as normal. However, the method as handled in this laboratory gave much higher results. Perhaps incubation for six hours, instead of placing in the refrigerator for eight hours, accounts for the difference. The analysis of the percentage changes in Groups I and II of the differential after the six-hour period can be used to show general trends only, because of the small size of the groups.

The hemophiliacs are separated according to clotting time and treatment. While the untreated hemophiliacs tend to give a decrease in Group I (average –25 per cent) and an increase in Group II (average +63 per cent), the transfused cases present the reverse: that is, an increase in Group I and a decrease in Group II. This is, perhaps, a step toward the normal distribution. The one hemophiliac treated with placental coagulant and the one treated with splenic extract and also the three with shorter clotting times, show the same type of variation as the transfused group.[14]

Several facts are apparent from this analysis: first, the menstruating women and all the hemophiliacs have a markedly delayed platelet disintegration rate; second, the differential changes are important as well as the actual disintegration rates. All the hemophiliacs vary considerably from the normal, but the untreated cases with long clotting times show the widest variance. The decrease in Group I with an increase in Group II suggests that the smaller

TABLE IV

DISINTEGRATION RATES—NORMAL WOMEN

(All platelet counts expressed in thousands)

CASE NO.	CLOTTING TIME[10]		TOTAL PLATELETS	DIFFERENTIAL COUNT		
				GROUP I	GROUP II	GROUP III
Nonmenstruating women						
5	10 min.	Initial	693	173	499	21
		After 6 hr. incubation	431	147	263	21
		Increase or decrease	−38%	−15%	−47%	
6	12 min.	Initial	302	184	118	0
		After 6 hr. incubation	210	57	153	0
		Increase or decrease	−30%	−69%	+30%	
8	9 min.	Initial	586	88	445	53
		After 6 hr. incubation	282	102	163	17
		Increase or decrease	−52%	+16%	−63%	
9	11 min.	Initial	828	248	580	0
		After 6 hr. incubation	494	213	281	0
		Increase or decrease	−40%	−14%	−52%	
		Initial	643	315	328	0
		After 6 hr. incubation	347	111	205	31
		Increase or decrease	−46%	−65%	−38%	
Menstruating women						
5	19 min.	Initial	426	111	298	17
		After 6 hr. incubation	592	142	414	36
		Increase or decrease	+39%	+28%	+39%	
		Initial	621	168	453	0
		After 6 hr. incubation	522	214	303	5
		Increase or decrease	−16%	+27%	−33%	
6	17 min.	Initial	485	218	267	0
		After 6 hr. incubation	413	124	252	37
		Increase or decrease	−15%	−43%	−6%	
7	19 min.	Initial	461	157	300	4
		After 6 hr. incubation	417	258	158	0
		Increase or decrease	−10%	+64 %	−47%	
8	15 min.	Initial	451	221	230	0
		After 6 hr. incubation	466	140	307	19
		Increase or decrease	+3%	−37%	+33%	
9	20 min.	Initial	504	222	257	25
		After 6 hr. incubation	513	308	205	0
		Increase or decrease	+2%	+39%	−20%	
		Initial	330	122	208	0
		After 6 hr. incubation	374	104	225	45
		Increase or decrease	+13%	−15%	+8%	

platelets have imbibed fluid and thus increased in size. Ferguson[15] has shown that the first stage in disintegration of the normal platelet is an absorption of fluid and consequent increase in size. Perhaps the process has stopped at this point instead of going on to complete breakdown or, in those subjects showing an increase in Group I, the process may have gone a step farther and resulted in the breaking up of the large forms into two or more smaller parts.

It seems obvious that transfusion and the treatment by splenic extract and placental coagulant have modified the abnormal differential somewhat, but not enough to change the disintegration rate. Clinical records indicate that

TABLE V

DISINTEGRATION RATES—HEMOPHILIACS

(All platelet counts expressed in thousands)

CASE NO.	CLOTTING TIME[10]		TOTAL PLATELETS	DIFFERENTIAL COUNT		
				GROUP I	GROUP II	GROUP III
No treatment						
15	2 hr.	Initial	748	524	210	14
		After 6 hr. incubation	545	262	261	22
		Increase or decrease	−27%	−50%	+24%	
		Initial	450	329	117	4.5
		After 6 hr. incubation	520	140	380	0
		Increase or decrease	+16%	−57%	+225%	
		Initial	1188	297	891	0
		After 6 hr. incubation	918	340	569	9
		Increase or decrease	−23%	+14%	−36%	
16	2½ hr.	Initial	640	365	269	6
		After 6 hr. incubation	498	204	289	5
		Increase or decrease	−22%	−44%	+7%	
17	1½ hr.	Initial	506	253	248	5
		After 6 hr. incubation	524	194	330	0
		Increase or decrease	+4%	−23%	+33%	
		Initial	252	126	101	25
		After 6 hr. incubation	477	167	305	5
		Increase or decrease	+89%	+32%	+202%	
18	2 hr.	Initial	1157	347	810	0
		After 6 hr. incubation	1124	180	944	0
		Increase or decrease	−3%	−48%	−16%	
21	17 min.	Initial	414	166	248	0
		After 6 hr. incubation	423	106	288	29
		Increase or decrease	+2%	−36%	+16%	
22	18 min.	Initial	317	101	216	0
		After 6 hr. incubation	335	101	230	4
		Increase or decrease	+6%	0%	+6%	
23	30 min.	Initial	639	256	377	6
		After 6 hr. incubation	530	159	366	5
		Increase or decrease	−17%	−38%	−3%	
Transfusion						
18		Initial	991	515	476	0
		After 6 hr. incubation	923	554	369	0
		Increase or decrease	−7%	+8%	−22%	
18		Initial	1114	345	758	11
		After 6 hr. incubation	1048	597	451	0
		Increase or decrease	−6%	+73%	−40%	
22		Initial	1441	476	922	43
		After 6 hr. incubation	1584	776	760	48
		Increase or decrease	+10%	+63%	−18%	
Placental coagulant						
19	4 hr.	Initial	974	127	847	0
		After 6 hr. incubation	991	268	723	1
		Increase or decrease	+2%	+111%	−15%	
Splenic extract						
20	5 hr.	Initial	450	270	167	13
		After 6 hr. incubation	515	412	98	5
		Increase or decrease	+14%	+52%	−41%	

TABLE VI

COMPARISON OF DIFFERENTIAL PLATELET COUNTS AFTER 6-HOUR DISINTEGRATION PERIOD

	AVG. DISINTEGRATION RATE	NUMBER OF SUBJECTS	GROUPS OF DETERMINATIONS	INCREASE AFTER 6 HOURS		DECREASE AFTER 6 HOURS		AVG. PERCENTAGE INCREASE OR DECREASE
				NUMBER	AVERAGE	NUMBER	AVERAGE	
					Per cent		*Per cent*	
Normal F (Nonmenstruating)	−41	4	5					
Group I				1	16	4	41	−30
Group II				1	30	4	50	−34
Normal F (Menstruating)	+2	5	7					
Group I				4	40	3	32	+ 9
Group II				3	27	4	26	− 3
Hemophiliacs (no treatment)	4.8	4	7					
Group I				2	23	5	44	−25
Group II				5	98	2	26	+62
Hemophiliacs (Transfused)	−1	2	3					
Group I				3	48	–	–	+48
Group II				–	–	3	27	−27
Hemophiliac (treated with placental extract)	+2	1	1					
Group I				1	111	–	–	+111
Group II				–	–	1	15	−15
Hemophiliac (treated with splenic extract)*	+14	1	1					
Group I				1	52	–	–	+52
Group II				–	–	1	41	−41
Hemophiliacs with maximum clotting time of 30″	−3	3	3					
Group I				(One showed no change)		2	37	−25
Group II				2	11	1	3	+ 6

*A protein-free splenic extract prepared by Wilson Laboratories, Chicago.

the treatment in the two cases made the bleeding less severe. The cases with short clotting times have the same type of differentials as the treated cases. This similarity in platelets may indicate the cause of their shorter clotting time.

It is very interesting that the menstruating individuals should also show a retarded disintegration rate. In the small series here no definite conclusions can be drawn concerning the differential distribution. Their clotting times fall within the same range as the mild hemophiliacs.

SENSITIVITY TO INTRADERMAL PLATELET INJECTIONS

For the past four years skin sensitivity tests with platelet preparations have been performed on subjects with normal clotting mechanisms, on hemophiliacs, and on patients with thrombopenic purpuras.

To prepare the platelet suspension, 10 c.c. of venous blood was drawn into a syringe containing 5 c.c. of 10 per cent sodium citrate. This was discharged into a flask containing another 5 c.c. of the citrate solution, making the final concentration 5 per cent. During the last year Olef's metaphosphate solution has been substituted for the citrate, since it seems to be a better platelet preservative. The blood was thoroughly chilled before centrifuging, and kept as cold as possible.

For injection the final washing of the suspension is performed with 0.85 per cent sodium chloride, and the platelets finally suspended in 3 c.c. of the saline. Sterile technique must be observed throughout.*

Table VII represents the results of intradermal injection of 0.1 c.c. of the sterile platelet suspension. The normal reaction consists of a wheal about 1/8 inch in diameter which can be definitely felt on palpation, with an area of redness 1/4 to 3/8 inch in diameter. Anything larger is considered to be abnormal and is designated as a hypersensitivity. Patients giving no reaction are designated as hyposensitive.

Nine subjects with normal clotting mechanisms and normal platelet counts are presented in Table VII. These give the reaction considered to be normal.

Eight untreated hemophiliacs gave absolutely negative reactions. This finding is very interesting when considered in the light of the retarded platelet disintegration rate of these patients, as given in Table V.

Subjects 31 through 36 are patients with thrombopenic purpuras, proved by history and prolonged observation to be of the chronic idiopathic type. In these cases an increased reaction was observed.

Subjects 37 through 41 are patients with secondary thrombopenias following measles. These may be divided into two classes. One class gave an increased, the other a negative, reaction during the purpura. Both returned to normal as the platelet count returned to normal.

An aplastic anemia case (Subject 42) gave a negative skin test. This patient may be assumed to have had an aplasia of the platelets.

The question of the interpretation of skin sensitivity to platelets and its use in clinical diagnosis becomes an interesting one. Three types of reactions have been demonstrated: the one occurring in the normal individual; an increased reaction occurring in the idiopathic thrombopenias and part of the secondary types; and the absolutely negative test demonstrated thus far only in hemophilia and a few secondary thrombopenias.

What determines sensitivity or the lack of it? It is known that all the blood components, including the platelets, are constantly being destroyed and replaced. Probably in the normal individual enough substances are released into the circulating blood, from the disintegrating platelets, to produce a slight skin sensitivity. The extreme opposite of the normal is represented by the idiopathic thrombopenia. Here the platelets are probably being destroyed rapidly, thus releasing an excessive amount of platelet substances into the blood. The fact that most of these cases after splenectomy have shown

*The lipid distribution of human platelets in health and disease.[9]

TABLE VII

SENSITIVITY TO INTRADERMAL PLATELET INJECTIONS

SUBJECT NO.	AGE	SEX	DIAGNOSIS	PLATELET COUNT*	SKIN SENSITIVITY TO PLATELETS	DISINTEGRATION RATE
5	23	F	Normal	693	Normal	–38
9	42	F	Normal	828	Normal	–40
24†	8	F	Mastoiditis	300	Normal	
25	4	M	Epilepsy	300	Normal	
26	5	F	Empyema	300	Normal	
27	6	F	Mastoiditis	250	Normal	
28	9	M	Mastoiditis	300	Normal	
29	12	M	Tuberculosis	250	Normal	
30	30	F	Normal	300	Normal	
15	13	M	Hemophilia	1188	Hypo-	22
16	7	M	Hemophilia	640	Hypo-	22
17	8	M	Hemophilia	506	Hypo-	3+
18	4	M	Hemophilia	1157	Hypo-	7+
19	9	M	Hemophilia	974	Hypo-	0.1
20	13	M	Hemophilia	450	Hypo-	14+
21	15	M	Hemophilia	414	Hypo-	2+
23	6	M	Hemophilia	639	Hypo-	17
			Thrombopenic purpura:			
31	10	M	Chronic idiopathic	50	Hyper-	
32	11	M	Chronic idiopathic	20	Hyper-	
33	8	M	Chronic idiopathic	20	Hyper-	
34	10	F	Chronic idiopathic	20	Hyper-	
35‡	11	F	Chronic idiopathic	24	Hyper-	
36	5	M	Chronic idiopathic	49	Hyper-	
37	2	F	Secondary thrombopenia	20	Hyper-	Post measles
37	2	F	Secondary thrombopenia (20 days later)	230	Normal	
38†	8	F	Secondary thrombopenia	19	Hyper-	Post measles
38†	8	F	Secondary thrombopenia (6 days later)	260	Normal	
39	2	M	Secondary thrombopenia	20	Hypo-	Post measles
39	2	M	Secondary thrombopenia (21 days later)	350	Normal	
40	3	F	Secondary thrombopenia	20	Hypo-	Post measles
40	3	F	Secondary thrombopenia (9 days later)	250	Normal	
41	2	M	Secondary thrombopenia	20	Hypo-	Post measles
41	2	M	Secondary thrombopenia (28 days later)	250	Normal	
42	5	F	Apastic anemia	10	Hypo-	

*All platelet counts expressed in thousands.

†Counts 24-30 and 38-42 were performed by a method which gives a lower count than Olef's.

‡The platelet samples used in this study were procured after splenectomy. Complete platelet analyses after splenectomy are presented.[9]

marked postoperative increases in the count, gradually returning to normal, would indicate that there is an actual hyperplasia of the platelet-forming tissue, thus leading to a still greater release of platelet substances. This would account for the increased skin sensitivity. The skin test upon an idiopathic thrombopenia should be a reliable basis on which to form a preoperative prognosis: the more marked the reaction, the more likely the patient will be to make a good recovery from the purpura after splenectomy. Any patient who appears to belong to this classification and gives a negative reaction should arouse a suspicion of an incorrect diagnosis.

The increased skin sensitivity found in part of the secondary thrombopenias is probably produced by the same mechanism as in the idiopathic types. The secondaries giving a negative reaction may be assumed to have a hypoplasia instead of an excessive destruction. In a hypoplasia or aplasia, where

few platelets are thrown into the blood stream, there will be a lack of disintegration products to stimulate a sensitivity. The fact that both of these secondary groups give a normal reaction after the platelet count has risen supports this theory. The skin test may, therefore, distinguish between excessive destruction and hypoplasia.

The negative reaction of the hemophiliacs is obviously not due to hypoplasia. Another factor is present in this disease which theoretically might lead to less than normal platelet destruction, namely, the decreased disintegration rate. Since the total count seems to follow the usual normal levels, that is, it is not increased in spite of the lessened disintegration, it is likely that the platelet-producing tissues are not as active as in the normal individuals. The skin sensitivity to platelets may prove to be a useful diagnostic test in hemophilia.

SUMMARY

A study of the total and differential platelet counts and disintegration rates, with changes in the differential at the end of the disintegration period, was made upon normal men and nonmenstruating women, normal women during menstruation, and hemophiliacs. The majority of the total counts in all groups were between 400 and 600 thousand. Acute colds seemed to cause a definite drop in the count. During menstruation the totals were moderately lowered.

When the count was high in normal individuals, the percentage of large platelets in the differential was higher, while in the hemophiliacs the proportion of large platelets was notably decreased. The disintegration rate determinations indicated a 30 per cent destruction as the lower limit of normal. Hemophiliacs and menstruating women gave a markedly retarded rate. The clotting time was delayed up to twenty minutes during menstruation, the normal with the method used being ten to twelve minutes. These points establish an interesting similarity between the blood of hemophiliacs and menstruating women.

Intradermal platelet injections were shown to produce a mild reaction in normal individuals whereas hemophiliacs gave a negative, and patients with chronic idiopathic thrombopenic purpuras an increased, reaction. One case of aplastic anemia with very few platelets gave a negative reaction. The secondary thrombopenias evidently presented a mixed group, some giving an increased reaction, others a negative. All reactions became normal, however, when the platelet count became normal.

The data obtained in this study indicate that platelet differential counts and disintegration rates, together with tests of skin sensitivity to platelets, may become important aids in diagnosis and prognosis.

REFERENCES

1. Chargaff, E., Bancroft, F. W., and Stanley-Brown, M.: Studies on the Chemistry of Blood Coagulation. The Chemical Constituents of Blood Platelets and Their Role in Blood Clotting, With Remarks on the Activation of Clotting by Lipids, J. Biol. Chem. **116**: 237, 1936.
2. Olef, I.: The Enumeration of Blood Platelets, J. Lab. & Clin. Med. **20**: 416, 1935.
3. Minot, G. R., and Lee, R. I.: The Blood Platelets in Hemophilia, Arch. Int. Med. **18**: 474, 1916.

4. Stübel, H.: Ultramikroskopische Studien über Blutgerinnung und Thrombocyten, Pflüger's Arch. f. d. ges. Physiol. **156**: 361, 1914.
5. Fonio, A.: Über die Gerinnungsfaktoren des Hämophileu Blutes, Mitt. a. d. Grenzgeb. d. Med. u. Chir. **28**: 313, 1914.
6. Howell, W. H., and Cekada, E. B.: The Cause of the Delayed Clotting in Hemophilic Blood, Am. J. Physiol. **78**: 500, 1926.
7. Kugelmass, I. N.: The Management of Hemorrhagic Problems in Infancy and Childhood, J. A. M. A. **99**: 895, 1932.
8. Olef, I.: Rate of Disintegration of Platelets, J. Lab. & Clin. Med. **22**: 128, 1936.
9. Erickson, Betty N., Lee, Pearl, Williams, H. H., and Avrin, I.: The Lipid Distribution of Human Platelets in Health and Disease, J. Clin. Investigation **18**: 81, 1939.
10. Lee, R. I., and Vincent, Beth: Coagulation of Normal Human Blood, Arch. Int. Med. **13**: 398, 1914.
11. Brakefield, J. L., and Malone, K.: Blood Platelet Numbers in Normal Men and Women, Ala. M. A. J. **5**: 373, 1936.
12. Genell, S.: Variations in the Number of Blood Platelets During the Menstrual Cycle, J. Obst. & Gynaec. Brit. Emp. **43**: 1124, 1936.
13. Olef, I.: Differential Platelet Count. Its Clinical Significance, Arch. Int. Med. **57**: 1163, 1936.
14. Eley, R. E., Green, A. A., and McKhann, C. F.: Use of Blood Coagulant Extract From Human Placenta in Treatment of Hemophilia, J. Pediat. **8**: 135, 1936.
15. Ferguson, J. H.: Observations on the Alterations of Blood Platelets as a Factor in Coagulation of the Blood, Am. J. Physiol. **108**: 670, 1934.

ACTION OF SODIUM THIOCYANATE UPON TISSUE OXYGEN CONSUMPTION*

Dale G. Friend, M.D., Boston, Mass., and
Roger W. Robinson, M.D., Worcester, Mass.

SINCE Pauli[1, 2] described the use of the thiocyanate ion in the treatment of hypertension, this drug has been used as a valuable therapeutic aid in several clinics both in this country and abroad. Favorable reports of the results in lowering blood pressure and relieving cerebral symptoms have appeared frequently in the literature. All investigators have noted toxic effects from the drug in various percentages of their cases. These consist of weakness, nausea, and vomiting, dermatitis, periods of hallucinations, motor aphasia, and even two fatalities with delirium, convulsions, coma, and death. The earlier use of the drug consisted of a daily dose between 1 and 2 gm. of either the sodium or potassium salt. Toxic symptoms were frequent with that dosage. After Barker[3] described the method of determining the thiocyanate concentration in the blood, it was possible to adjust the dose to certain blood concentrations in each patient, and the more serious toxic symptoms from the drug have been experienced less frequently.

With the wide use of this drug, it is surprising that so little is known about its pharmacology and mode of action in lowering blood pressure. Claude Bernard[4] in 1857 described experiments in which he gave large doses of thiocyanate intravenously to dogs and came to the conclusion that the drug was a direct smooth muscle poison. It abolished muscular activity without

*From the Department of Biochemistry of the Harvard Medical School and the Medical Clinics of the Peter Bent Brigham Hospital, Boston.
Received for publication, October 22, 1938.

producing any sensory change. Pauli[1, 2] and Bentley and LeRoy[5] thought that the beneficial results in arteriosclerosis were due to the power of thiocyanate to dissolve calcareous deposits in the arteriosclerotic areas in the arteries. Logefiel[6] opposed this viewpoint because of the prompt drop in blood pressure produced by the drug and the fact that the drop was not permanent. Pauli[1, 2] also suggested that the fall in blood pressure might be due to a "decrease in the energy of the heart muscle." In this regard Palmer, Silver, and White[7] noticed a softening in the force of the heartbeat in some hypertensive cases receiving thiocyanate. Nichols[8] believes that in toxic doses, thiocyanate is a direct muscle poison, abolishing muscle activity. It is known that the thiocyanate ion diffuses equally into all extracellular fluids with the same ease as urea. Because of this property, it has been used by Crandall and Anderson[9] as a method of determining the total amount of extracellular fluid. Takacs[10] believed that thiocyanate was a powerful vagal stimulant and explained the drop in blood pressure on this basis. Westphal and Blum[11] suggested that thiocyanate "increases the permeability of the arterioles and decreases their tonus."

From this discussion it is evident that several theories have been advanced for the action of thiocyanate, but none is substantiated by sufficient evidence to warrant its acceptance. One of us, in studying a large group of cases with essential hypertension with this drug, was impressed by the frequent statement by patients that they were weak, tired, lazy, or lacked energy. Some volunteered the information that their skin was unusually dry and they noticed cold weather more than previously. They exhibited symptoms suggestive of depression of their metabolism.

With these observations in mind we studied the action of sodium thiocyanate upon the oxygen consumption of normal rat liver in human serum. Blood was drawn from hypertensive patients receiving no medication, and the serum was prepared free of carbon dioxide as described elsewhere.[1] A portion of the serum was used for control experiments without thiocyanate. Sodium thiocyanate in varying amounts was added to the remaining portion. The QO_2 was determined according to our previously described technique.[1]

From Table I it can readily be seen that the drug does depress the oxygen consumption of normal tissue. Of course, the higher concentrations that were studied are far above the therapeutic and well into the toxic range. The lower levels, however, are occasionally seen in patients.

The limiting concentrations, the effect of which can be detected with this procedure, appear to be between 20 and 30 mg. per 100 c.c.

It is interesting to point out that sodium thiocyanate depresses the oxygen consumption of rat liver almost as much as does a similar dose of chloral hydrate.[2] The liver tissue so depressed by thiocyanate, we have found from some preliminary experiments, is capable of being stimulated by methylthionine chloride when it is suspended in human serum.

We next studied the action of serum from patients receiving sodium thiocyanate upon oxygen consumption of rat liver. The tissue, suspended in the serum, was compared with controls suspended in serum from another

TABLE I

ACTION OF SODIUM THIOCYANATE ON OXYGEN CONSUMPTION OF RAT LIVER

NUMBER	MG. NaSCN PER C.C.	QO_2 NO NaSCN	QO_2 WITH NaSCN	% REDUCTION OF O_2 CONSUMPTION
1	8.0	−14.7	− 9.2	−37
2	4.0	−14.7	−10.5	−28
3	2.0	−11.3	− 9.4	−17
4	0.3	−14.0	−11.9	−15
5	0.2	−12.1	−10.8	−11
6	0.15	−12.1	−11.6	− 5
7	0.15	−10.2	−10.2	− 0

hypertensive patient who was not receiving sodium thiocyanate. The sodium thiocyanate of the serum was checked by chemical analysis. The drug content varied from 8 to 22 mg. per 100 c.c. These amounts fall in the lower limit of our method, thus making it doubtful whether we would be able to detect the effect of the presence of the drug in serum containing less than 20 mg. per 100 c.c. (see Table II).

These results, although indicating that there is some decreased oxygen consumption, are not striking, in view of the fact that variations in the oxygen consumption of less than 10 per cent are probably of questionable significance. We are at present unable to say whether there is a definite decreased oxygen consumption at the therapeutic levels or not. Nevertheless, in concentration

TABLE II

ACTION OF SODIUM THIOCYANATE SERUM ON OXYGEN CONSUMPTION OF RAT LIVER

PATIENT	BLOOD NaSCN MG. %	QO_2 HYPERTENSIVE CONTROL SERUM	QO_2 HYPERTENSIVE NaSCN SERUM	% DECREASE IN O_2 UPTAKE
1	10.6	−13.6	−12.3	− 9.8
2	12.1	−10.2	− 9.7	− 4.9
3	10.3	−12.7	−12.4	− 2.4
4	9.5	−11.3	−10.6	− 6.2
5	8.0	−12.1	−11.8	− 2.5
6	9.6	− 9.7	−10.0	− 3.1
7	22.0	−10.0	− 8.5	−15.0

ranges where the action of this drug can be studied using our technique, it apparently exerts a definite depressing effect upon tissue respiration. It should be remembered that in our experiments the tissue is in contact with the serum plus thiocyanate only one hour, while the cells in the patient are constantly exposed to it for weeks. This depressing effect is analogous to the effect of traces of potassium cyanide and chloral hydrate. Just where in the oxidation chain thiocyanate exerts its influence we are unable to say. It does not interfere with the stimulating action of methylthionine chloride. However, one patient who exhibited toxic symptoms from the drug was not relieved when methylthionine chloride was given orally for forty-eight hours.

SUMMARY

1. Oxygen consumption of rat liver cells is depressed by the thiocyanate ion when added to serum in vitro.

2. Serums from patients receiving thiocyanate and exhibiting concentrations of the drug from 8 to 22 mg. per 100 c.c. do not appear to depress the oxygen consumption of rat liver suspended in them.

3. Methylthionine chloride, when given orally, does not exert any ameliorative effect.

REFERENCES

1. Pauli, W.: München. med. Wchnschr. **50:** 153, 1903.
2. Pauli, W.: Physical Chemistry in the Service of Medicine. Translated by M. H. Fisher, New York, 1906, Wiley & Sons.
3. Barker, M. H.: Blood Cyanates in the Treatment of Hypertension, J. A. M. A. **106:** 762, 1936.
4. Bernard, C.: Lecons sur les effets des substances toxiques et medicamentenses **7:** 1857.
5. Bentley, W., and LeRoy, B. R.: The Solvent Action of the Sulfocyanates Upon the Lime and Magnesium Deposits in the System, New York M. J. **88:** 210, 1908.
6. Logefiel, R. C.: Observations on the Use of Potassium Sulfocyanate in the Treatment of Essential Hypertension, Minnesota Med. **12:** 151, 1929.
7. Palmer, R. S., Silver, L. S., and White, P. D.: Clinical Use of Potassium Sulfocyanate in Hypertension, New England J. Med. **201:** 709, 1929.
8. Nichols, J. B.: Pharmacologic and Therapeutic Properties of Sulfocyanate, Am. J. M. Sc. **170:** 735, 1925.
9. Crandall, L. A., and Anderson, M. X.: Estimations of the State of Hydration of the Body by Amount of Water Available for Solution of Sodium Thiocyanate, Am. J. Digest. Dis. & Nutrition **1:** 126, 1934.
10. Takacs, L.: Thiocyanates, Ztschr. f. d. ges. exper. Med. **50:** 432, 1926.
11. Westphal, K., and Blum, R.: Die Rhodantherapie des genuinen Arteriellen Hochdrucks und irhe theoretische Begrundung, Deutsches Arch. f. klin. Med. **152:** 331, 1926.

LIVER DYSFUNCTION AS A POSSIBLE CAUSATIVE FACTOR IN RENAL LITHIASIS*

A Clinical Investigation on 39 Patients

William J. Ezickson, A.B., M.D., Philadelphia, Pa.

INTRODUCTION

THESE clinical investigations concerning the possible role of the liver in the pathogenesis of renal lithiasis, we believe, are the first to have been undertaken. During the past ten months, studies have been made in the Calculus Research Clinic at the Pennsylvania Hospital, Philadelphia. Liver function tests were made on individuals who have or have had renal or ureteral calculi. We believe the findings are intensely interesting and warrant bringing them to your attention. It is possible this may open up a new avenue to the approach of the problem of urinary lithiasis.

Previous Studies and Findings That Lead to This Investigation.—The author,[1] in collaboration with Dr. J. B. Feldman, presented before the American Medical Association, at Atlantic City, in June, 1937, a paper which dealt with the incidence of vitamin A deficiency in a group of individuals with urinary lithiasis. It was shown in this study that 96 per cent of these patients showed a vitamin A deficiency. It was also shown that the giving of vitamin A concentrate by mouth in large amounts over a period of six to nine months did not alter this deficiency. This suggested to us the possibility that in individuals with urinary lithiasis, there was either a lack of assimilation of vitamin A, or if assimilation did take place, a lack of utilization due to some unknown disturbance of metabolism. Since the liver is known to be the storehouse for this vitamin, we decided to turn our attention to the liver and determine, if possible, if there was any disturbance in the liver function of these patients.

Liver Function Test Used in These Studies.—We used the bromsulphalein test in making these studies. We believe this test to be as reliable as any, and in a recent article on the use and interpretation of tests for liver function, Snell and Magath[2] state: "In types of disease of the liver not associated with jaundice, information gained from the study of retention of bromsulphalein is as reliable as that which can be gained in any other way, and that under these conditions, other tests give chiefly confirmatory evidence."

In most of the cases we injected 5 mg. of the dye per kilogram of body weight, as suggested by O'Leary, Greene, and Rowntree.[3] In a few cases we

*From the Calculus Research Clinic, Department of Urology, Pennsylvania Hospital, Philadelphia.

Presented before a combined meeting of the Philadelphia Urological Society and the New York Urological Society, Philadelphia, April 21, 1938.

Received for publication, October 25, 1938.

injected 2 mg. of the dye per kilogram of body weight. This latter dosage was proposed at first, but later it was shown that the test could be improved by injection of the larger dose (5 mg. per kg. of body weight).

Analysis of Results of Bromsulphalein Test.—Group 1 was given 5 mg. per kg. nonfasting, and a thirty-minute specimen was taken. In this group, which consisted of 30 cases, 15 showed dye retention of over 10 per cent, and of these 15, 9 had retention of 14 per cent to 20 per cent, 4 had 20 per cent to 50 per cent, and 2 had over 50 per cent. Table I shows these results in detail.

TABLE I

BROMSULPHALEIN RETENTION

Group 1. 5 mg. per kg. of the Dye Injected, 30-Minute Specimen, Nonfasting

CASE	PER CENT	CASE	PER CENT
1	42	16	18
2	15	17	10
3	4	18	16
4	3	19	43
5	19	20	8
6	21	21	10
7	0	22	10
8	6	23	Traces
9	9	24	18
10	Traces	25	2
11	14	26	22
12	16	27	4
13	Traces	28	16
14	16	29	58
15	60	30	7

Group 2 was given 5 mg. per kg., on a fasting stomach, and a thirty-minute specimen was taken. In this group, which consisted of 10 cases, 9 had retention of the dye over 10 per cent, of which 5 had retention of 13 per cent to 20 per cent, and 4 had 20 per cent to 40 per cent. Table II shows these results in detail.

TABLE II

BROMSULPHALEIN RETENTION

Group 2. 5 mg. per kg. of the Dye Injected, 30-Minute Specimen, Fasting

CASE	PER CENT	CASE	PER CENT
2	26	32	16
17	13	33	40
19	1	34	13
24	17	35	32
31	14	39	38

Group 3 was given 2 mg. per kg., on a fasting stomach, and a thirty-minute specimen was taken. In this group, which consisted of 7 cases, 3 had retention of 3 per cent to 5 per cent, and one was over 20 per cent. Table III shows these results in detail.

Certain cases appear in more than one group, and can be followed by the particular case numbers, which would be the same in each group. This was done to check one method of giving the dye against another, and the results obtained.

TABLE III

BROMSULPHALEIN RETENTION

Group 3. 2 mg. per kg. of the Dye Injected, 30-Minute Specimen, Fasting

CASE	PER CENT	CASE	PER CENT
1	5	36	1
12	27	37	3
26	1	38	3
29	1		

TABLE IV

CASE	AGE	SEX	CALCULUS AT PRESENT	LOCATION	PREVIOUS OPERATIONS	CALCULUS PASSED SPONTANEOUSLY
1	46	M	Yes	Lt. kidney	1932 Rt. ureterolithotomy	No
2	49	F	Yes	Rt. kidney	1925 Rt. pyelolithotomy 1931 Rt. pyelolithotomy 1935 Lt. nephrectomy	No
3	34	F	Yes	Rt. kidney	1936 Rt. ureterolithotomy	No
4	22	F	Yes	Rt. ureter	None	Yes
5	44	M	No		None	Yes
6	49	F	No		1932 Rt. nephrectomy	No
7	34	M	No		1934 Rt. nephrectomy	No
8	33	M	Yes	Lt. kidney	1922 Bladder stone-cystotomy 1925 Rt. nephrectomy 1932 (Sept.) Lt. ureterolithotomy 1932 (Oct.) Lt. ureterolithotomy	Yes
9	61	F	Yes	Lt. ureter	1932 Rt. ureterolithotomy	No
10	14	M	No		1937 Rt. nephrectomy	No
11	55	M	No		1937 Lt. nephrectomy	No
12	48	F	No		1934 Lt. nephrectomy	No
13	35	M	Yes	Rt. kidney pelvis	None	No
14	28	M	Yes	Lt. kidney	1925 Lt. pyelolithotomy 1934 Rt. nephrectomy	No
15	44	M	No		None	Yes
16	36	M	Yes	Lt. kidney	1938 Lt. nephrolithotomy	Yes
17	27	M	Yes	Both kidneys	1936 Rt. ureterolithotomy	Yes
18	37	F	Yes	Lt. ureter	None	Yes
19	27	F	No		1936 Rt. ureterolithotomy	No
20	39	F	No		1937 Lt. pyelolithotomy	Yes
21	41	M	No		1936 Lt. nephrectomy	No
22	32	F	No		1927 Rt. pyelolithotomy 1937 Rt. pyelolithotomy	No
23	41	M	Yes	Rt. ureter	None	No
24	45	F	Yes	Both kidneys	1933 Lt. pyelolithotomy 1935 Rt. ureterolithotomy	No
25	42	M	Yes	Rt. kidney	1936 Rt. nephrectomy 1936 Lt. pyelolithotomy	No
26	48	F	Yes	Lt. kidney	None	Yes
27	40	F	No		1937 Rt. ureterolithotomy	No
28	31	M	No		1937 Lt. nephrolithotomy	No
29	42	M	Yes	Lt. kidney	None	Yes
30	36	F	No		1937 Rt. ureterolithotomy	No
31	28	F	No		1938 Lt. nephrectomy	No
32	46	M	No		1925 Lt. pyelolithotomy	Yes
33	47	F	No		1928 Lt. pyelolithotomy	Yes
34	42	M	Yes	Lt. ureter	None	No
35	42	F	No		None	Yes
36	20	F	Yes	Lt. kidney Rt. ureter	None	Yes
37	20	F	Yes	Lt. kidney	None	No
38	41	M	Yes	Rt. ureter	None	Yes
39	39	F	Yes	In stump of rt. ureter	1930 Rt. pyelolithotomy 1931 Rt. nephrectomy (subcapsular)	No

It is most important to bear in mind that none of these patients had any clinical evidence of liver disease. Under these circumstances, we believe the figures, showing such a large percentage of these patients having a high percentage of dye retention, to be rather significant.

Data Pertaining to the History of Calculi in These Patients.—All of the individuals studied in this investigation have or have had renal or ureteral calculi. The details concerning their calculi history are shown in Table IV. This table shows (1) age; (2) sex; (3) whether or not the patient has calculus at the present time; and (4) if present, its location; (5) previous operations, renal or ureteral, if any; (6) whether or not calculus passed spontaneously. An analysis of these patients shows the following: Average age 38 plus; 19 males, 20 females; 21 have calculi at the present time, located as follows: 4 in the right kidney, 7 in the left kidney, 2 bilateral renal calculi, 4 in the right ureter, 3 in the left ureter, 1 in the left kidney and right ureter.

Twenty-six have had renal or ureteral surgery which included: 4 pyelolithotomies; one nephrolithotomy; 7 ureterolithotomies; 7 nephrectomies; 4 pyelolithotomies and nephrectomies; one had 2 pyelolithotomies; one had 2 pyelolithotomies and a nephrectomy; one had a cystotomy, 2 ureterolithotomies and a nephrectomy. Fifteen have passed calculi spontaneously.

The case numbers in this table correspond to the same case numbers shown in Tables I, II, and III.

Studies for Vitamin A Deficiency.—Of this group of 39 patients, 34 were studied for vitamin A deficiency. Thirty-two were found deficient and 2 were normal. The studies for vitamin A deficiency were made by means of the dark adaptation test, using the Feldman Adaptometer. The method and instrument have been described in recent literature.[4-6] We used this method, as it is held to be the most reliable and also the most scientific. Yudkin,[7] in a recent article, states that this method is the most satisfactory.

The fact that 94 per cent of the tested group showed vitamin A deficiency is very significant when compared to the observations made by Moore and Ellison.[8] They analyzed the amount of vitamin A concentration in the livers of 1,000 adults that came to autopsy. The lowest concentrations were found in those patients who died of kidney and bladder infections.

SUMMARY

1. Studies of the liver function were made on 39 individuals who have or have had renal or ureteral calculi.

2. The bromsulphalein test was used to determine the liver function.

3. In group 1 (given 5 mg. per kg., thirty-minute specimen, nonfasting) 50 per cent showed retention of the dye, ranging from 14 per cent to 60 per cent.

In group 2 (given 5 mg. per kg., thirty-minute specimen, fasting) 90 per cent showed retention of the dye, ranging from 13 per cent to 40 per cent.

4. Thirty-four patients in this group were studied for vitamin A deficiency and 32 (94 per cent) showed a deficiency.

5. The findings in this investigation lead us to believe that there is a close relationship between liver dysfunction, vitamin A deficiency, and renal lithiasis. This should open up a new avenue to the approach of the problem of the pathogenesis of renal calculi, and we believe this should stimulate further investigations along this line.

I wish to express my thanks to Dr. John T. Bauer, Director of the Clinical Laboratory of the Pennsylvania Hospital, and his associate, Dr. William Wolff, for their cooperation in making these studies.

REFERENCES

1. Ezickson, William J., and Feldman, J. B.: Signs of Vitamin A Deficiency in the Eye Correlated With Urinary Lithiasis, J. A. M. A. **109**: 1706, 1937.
2. Snell, Albert M., and Magath, Thomas B.: The Use and Interpretation of Tests for Liver Function, J. A. M. A. **110**: 167, 1938.
3. O'Leary, P. A., Greene, C. H., and Rowntree, L. G.: Diseases of the Liver. VIII. The Various Types of Syphilis of the Liver With Reference to Tests for Hepatic Function, Arch. Int. Med. **44**: 155, 1929.
4. Feldman, J. B.: Dark Adaptation as a Clinical Test, Arch. Ophth. **15**: 1004, 1936.
5. Feldman, J. B.: Dark Adaptation as a Clinical Test. Further Studies, Arch. Ophth. **17**: 648, 1937.
6. Feldman, J. B.: Instrument for the Qualitative Study of Dark Adaptation, Arch. Ophth. **18**: 821, 1937.
7. Yudkin, A. M.: Vitamins in Treatment and Prevention of Ocular Diseases, Arch. Ophth. **19**: 365, 1937.
8. Current Comment: Vitamin A Reserves in Health and Disease, J. A. M. A. **109**: 590, 1937.

ELECTROCARDIOGRAPHIC CHANGES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF MAGNESIUM SULFATE*

II. An Experimental Study on Dogs

T. R. Van Dellen, B.S., M.D., and J. Roscoe Miller, M.S., M.D.,
Chicago, Ill.

In a previous study[1] it was shown by means of electrocardiographic studies that magnesium, when injected intravenously, has a definite effect upon the cardiac conduction system. This effect consisted of an early acceleration followed by slowing, with delay in the auriculoventricular and ventricular conduction time and increased excursion of all complexes. This effect is not permanent.

Continued study seemed indicated to determine whether this was a direct effect upon the conductive system and what part, if any, the vagus nerve had in the result.

Di Macco[2] was of the opinion that the action of magnesium on cardiac activity was dependent on vagal effect. His conclusions were based on blood pressure readings. He further observed that the effect was most pronounced when atropine was given after magnesium had been injected.

*From the Department of Medicine, Northwestern University Medical School.
Received for publication, November 4, 1938.

METHODS

As in previous experiments[1] the unanesthetized dog was placed on the left side and electrodes attached to the right front and left rear legs. Electrocardiograms were made on a string galvanometer type machine. Both magnesium and atropine were injected into the right saphenous vein.

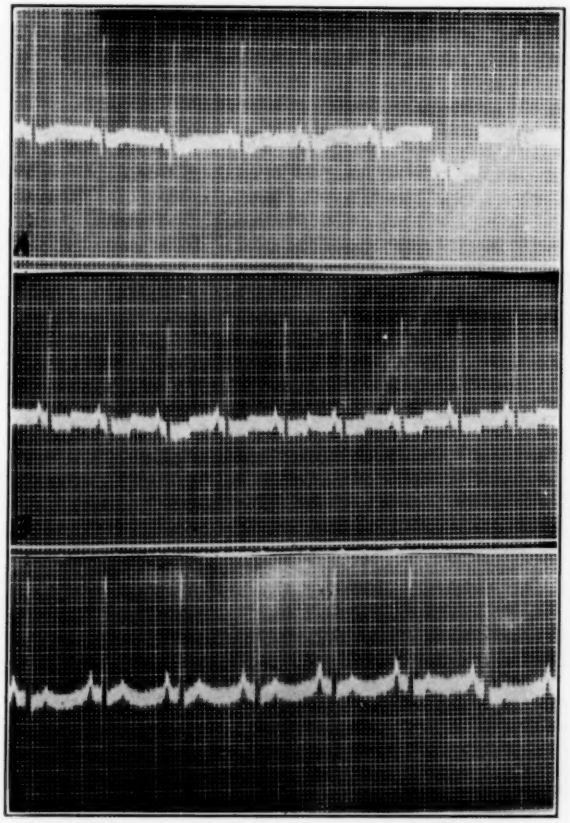


Fig. 1.—Dog B (14 kg.). *A*, Normal graph. *B*, After injection of atropine sulfate. *C*, After injection of 10 c.c. of a 20 per cent solution of magnesium sulfate.

RESULTS

Experiment 1.—In an attempt to determine the effect of atropine alone, 3 dogs were injected with small, so-called stimulating doses, 0.01 mg. per kg., and then large paralyzing doses, 0.2 mg. per kg.[3] Electrocardiograms were made during injection and at intervals for three hours afterward. The results were similar to those described by Halsey,[4] the changes in rate being the most significant.

Experiment 2.—Three dogs were given a large dose of atropine sulfate (0.2 mg. per kg. body weight). The solution was of such concentration that 1 c.c. contained 0.4 mg. of atropine. Immediately following injection, one dog was given 10 c.c. of a 20 per cent solution of magnesium sulfate, while the other two were given 20 c.c. of the same solution. The magnesium sulfate in these experiments was injected intravenously at the rate of 2 c.c. per minute.

The effect was the same as that seen when magnesium sulfate was given without a preceding injection of atropine (Fig. 1). The rate was slower at first, but later accelerated. The P-R interval increased from 0.09 to 0.12 second, and the QRS complex from 0.04 to 0.06 second. The height and contour of the P- and T-waves were changed in the same manner as in the unatropinized animals.

All of these results became more pronounced with the increased dose of magnesium.

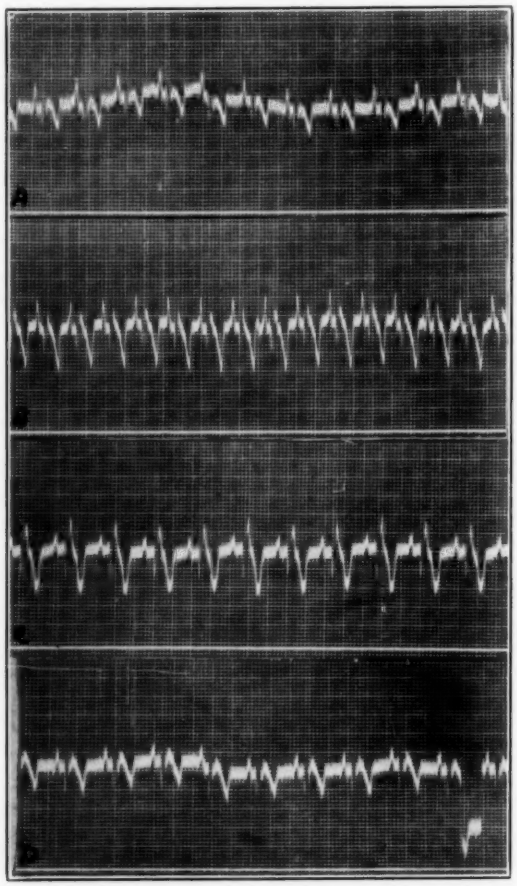


Fig. 2.—Dog V (10.7 kg.). *A*, Normal graph. *B*, After bilateral vagotomy. *C*, After injection of magnesium sulfate solution. *D*, After injection of atropine sulfate.

Experiment 3.—One animal was anesthetized and the vagi were sectioned. Four hours later, when recovery was apparently complete, an electrocardiogram showed no change when compared with one made previous to operation, except that the rate was increased. Ten cubic centimeters of a 20 per cent solution of magnesium sulfate was then injected intravenously and records made during and after the injection.

The result (Fig. 2) corresponded to that obtained in animals with unsectioned vagi. The P-R interval was increased from 0.09 to 0.14 second, and

the QRS complex from 0.04 to 0.08 second. A large dose of atropine, 0.2 per kg., was then given, with no demonstrable results.

SUMMARY

These experiments indicate that the effect of magnesium upon the cardiac conductive system is not central but direct.

REFERENCES

1. Miller, J. Roscoe, and Van Dellen, T. R.: Electrocardiographic Changes Following the Intravenous Administration of Magnesium Sulfate. An Experimental Study on Dogs, J. LAB. & CLIN. MED. **23**: 914, 1938.
2. Di Macco, G.: The Effect of Atropine on the Hypotensive Action of Magnesium, Riv. di. pat. sper. **5**: 393, 1930.
3. Pelcher, J. D., and Sollmann, Torald: Quantitative Studies of Vagus Stimulation and Atropine, J. Pharmacol. **5**: 317, 1914.
4. Halsey, J. T.: The Digitalized Dog's Heart as Affected by Amyl Nitrate or Atropine Studied Electrocardiographically, J. Exper. Med. **25**: 729, 1917.

LABORATORY METHODS

SODIUM FERROCYANIDE EXCRETION IN THE ESTIMATION OF RENAL FUNCTION*

MILTON PLOTZ, M.D., F.A.C.P., AND MARGARETHE ROTHENBERGER, M.D.
BROOKLYN, N. Y.

WITH THE TECHNICAL ASSISTANCE OF EDGAR FERGUSON, AND
VICTOR GINSBURG, M.D.

THE use of sodium ferrocyanide in estimating renal function was first proposed by Stieglitz and Knight[1] in 1934. The present study was undertaken on the wards of the Long Island College Division of the Kings County Hospital as part of a project for estimating the clinical value of kidney function tests. Two-hour phenolsulfonphthalein, fractional phenolsulfonphthalein, concentration tests, urea clearances, detailed analyses of the blood, urinary and clinical data were done in combinations of three or more of these on each patient and in most cases all the tests were performed. This report will be concerned only with the results obtained with sodium ferrocyanide; other details of the project will be reported in detail elsewhere.

Stieglitz and Knight,[1] on the basis of experimental work done on the rabbit, proposed that their test be considered essentially as a measure of pure glomerular filtration. Van Slyke, Hiller, and Miller[2] found additional evidence to support the view of Gersh and Stieglitz[3] that, in the dog and rabbit at least, sodium ferrocyanide excretion is a true measure of glomerular filtration. That these conclusions cannot be carried over to man is indicated by Miller and Winkler,[4] whose data suggest that, in contrast to the situation in the dog, 40 per cent of the ferrocyanide excreted by the human glomerulus is reabsorbed by the tubule. Of course, this finding does not mean that sodium ferrocyanide is without value as a renal function test; it means only that, in any case, it cannot be accepted only as a test of glomerular function.

Method.—The method of Stieglitz and Knight[1] was followed in detail but was occasionally supplemented by a modification in titration in order to gain added accuracy in determining the end point. The drug, generously supplied by the Abbott laboratories in ampoules containing 0.25 gm. of the anhydrous

*From Kings County Hospital, Long Island College Division, the medical services of Dr. Tasker Howard and Dr. Carl H. Greene.

This study was made possible by a generous grant from the Harriet Weinbaum Foundation.

Clinical studies in renal function No. 4 from the Long Island College of Medicine.

Received for publication, July 29, 1938.

salt, was dissolved in 10 c.c. of sterile distilled water. In some cases, in order to compare the urea clearance and the ferrocyanide test, the two were run simultaneously. The urine was collected at fifteen, thirty, sixty, and one hundred twenty minutes. It was found that urine passed during the third hour usually contained very little ferrocyanide, and the collection of a one-hundred-eighty-minute specimen was abandoned. The titration was done twice; first, with 0.4 per cent copper sulfate, as suggested by Stieglitz and Knight, and again, as a check, with 0.1 per cent copper sulfate. The latter modification was suggested by the finding that, when using a small sample, it is difficult to get an accurate reading with the amount of 0.4 per cent copper sulfate used. A quicker and simpler method of titration was used as a supplement in some of the later cases. Standards were set up containing the maximum and minimum expected percentages of ferrocyanide (diluting from a weighed standard) in about ten steps. To 10 c.c. of these standard solutions was added 1 c.c. of 0.5 per cent ferric chloride. This was repeated with the unknown. These standards will keep for several days; the color comparison is easily made and corrected for turbidity as in the standard phenolsulfonphthalein procedure.

RESULTS

A. *Normals.*—Seventy-eight young adults on the medical wards were carefully selected for their freedom from any signs of kidney insufficiency. Most of them were suffering from peptic ulcer or arthritis, although there was an occasional drug addict or diabetic. In 17 cases, the ferrocyanide test indicated markedly diminished renal function. In all of these patients excretion of phenolsulfonphthalein, urea clearance, and urine concentration tests were perfectly normal. In other words, there was a 22 per cent error with ferrocyanide. In the same series there was a 14 per cent error with the two-hour phenolsulfonphthalein test. In only one case did both the phenolsulfonphthalein and the ferrocyanide excretions indicate diminished renal function in the same patient. In this case, a repetition of the phenolsulfonphthalein test gave normal figures; this was not true for ferrocyanide. The thirty-minute phenolsulfonphthalein test was in error in only 8 per cent of these normals and, in all but one case, normal figures were obtained when the test was repeated. At this point it should be emphasized that every test for kidney function now in common clinical use may occasionally give an erroneous picture in normal patients and later show normal function when repeated under somewhat different conditions or at another time of the day. It is well to bear this point in mind before concluding, on the evidence of a single unrepeated test, that a patient is suffering from impaired renal function.

B. *Known Kidney Disease.*—There were 19 patients with known kidney disease in whom the urea clearance was definitely diminished and in whom there was unmistakable clinical evidence of nephritis or nephrosclerosis. In two of these, there was normal ferrocyanide excretion even when the test was repeated. In one patient there was a subacute hemorrhagic nephritis with a blood pressure at rest of 150/95, hematuria, and a low urea clearance. The excretion of ferrocyanide and phenolsulfonphthalein (both thirty minutes and two hours)

were within normal limits. In the second patient, one of advanced nephrosclerosis, urine concentration tests, urea clearance, phenolsulfonphthalein, and blood urea agreed, but there was normal ferrocyanide excretion. In this case it appears that the ferrocyanide test gave a definitely erroneous impression; the first case is more difficult to interpret. On the whole, we felt that the urea clearance gave the most accurate picture of the case. The subsequent course of the patient could not be followed, so it is impossible to state whether or not she went on to more advanced impairment of renal function.

C. *Hypertension.*—Stieglitz and Knight analyzed a series of 11 cases of hypertension, with adequate cardiac efficiency and no urinary evidence of kidney injury. They found that the ferrocyanide excretion was diminished more often and more markedly than that of phenolsulfonphthalein. This they took as evidence of the greater sensitivity of the ferrocyanide test to changes in the glomerular circulation, which was to be expected in view of the predominantly glomerular excretion of ferrocyanide and the tubular excretion of phenolsulfonphthalein. We investigated a series of 27 similar cases. Fifteen had normal renal function tests; the ferrocyanide excretion was below normal in 12, being considerably reduced in 3. Phenolsulfonphthalein was reduced to a much smaller degree and in only 7 cases, all of which had lowered ferrocyanide excretion. The urea clearance was diminished in only 8 of the 27 patients. These results, although not based on enough cases to draw any conclusions, would seem to confirm the opinion of Stieglitz and Knight that ferrocyanide excretion is diminished earlier in hypertension than other tests of renal function. One should not, on the basis of these results, hasten to assume without further evidence that glomerular inefficiency is the cause of the hypertension in the cases which show renal function impairment.

D. *Heart Failure.*—There were 21 cases of cardiac decompensation. Fifteen showed moderate to severe renal involvement, as shown by the ferrocyanide test. All of these showed improvement as the cardiac failure improved.

Reactions.—There were 251 injections in 145 patients. Fourteen times there were reactions, 6 per cent of all injections, 9.6 per cent of all patients. Where there was a reaction, the injection was never repeated. Where there was no reaction to the first injection, there was no reaction to subsequent ones. In 7 patients there was nausea or vomiting; in 6 there were weakness and dizziness or syncope; one had an anuria lasting eight hours. The most severe reaction occurred in the normal control group. The patient developed a gross hematuria which continued for three hours, after which the urine was normal in every respect.

CONCLUSIONS

1. The sodium ferrocyanide test was performed 251 times on 145 patients.

2. It was not so reliable as the fractional phenolsulfonphthalein test or the urea clearance test in revealing normal renal function in a control group.

3. It was slightly less reliable in revealing impairment of kidney function in renal disease.

4. It was somewhat more sensitive in detecting early renal damage in hypertension than other function tests.

5. Reactions occurred in 9.6 per cent of cases. None were fatal or permanent.

6. For routine use, the test has no advantage over the fractional phenolsulfonphthalein or urea clearance.

REFERENCES

1. Stieglitz, E. J., and Knight, A. A.: Sodium Ferrocyanide as a Clinical Test of Glomerular Efficiency, Preliminary Report, J. A. M. A. **103**: 1760, 1934.
2. Van Slyke, D. D., Hiller, A., and Miller, B. F.: The Clearance, Extraction Percentage and Estimated Filtration of Sodium Ferrocyanide in the Mammalian Kidney. Comparison With Inulin, Creatinin and Urea, Am. J. Physiol. **113**: 611, 1935.
3. Gersh, I., and Stieglitz, E. J.: Histochemical Studies in the Mammalian Kidney. I. The Glomerular Filtration of Ferrocyanide in the Rabbit and Some Related Problems, Anat. Rec. **58**: 349, 1934.
4. Miller, B. F., and Winkler, A.: Ferrocyanide Excretion in Man, J. Clin. Investigation **15**: 489, 1936.

A DEVICE FOR VIEWING PRECIPITIN REACTIONS*

ELIZABETH M. KANNE, M.A., AND JANET R. MCCARTER, PH.D.
MADISON, WIS.

PRECIPITIN reactions have usually been read against a window bar with sunlight illumination, or against a background of black cloth with an electric light. Under these conditions readings by the same individual on the same antigen-antibody system have varied considerably with the intensity of the sunlight, and even under apparently similar conditions readings by two persons have varied slightly. Readings for rings or turbidity have been consistent when made with the use of the lighting box, designed for us by Dr. W. R. Kanne, and illustrated in Fig. 1. Although other devices[1] have been suggested, this one possesses features of simplicity in both construction and equipment. It has also been found satisfactory for reading Kahn tests.

The principal feature of the box is that no direct light from the source of illumination can reach the eye. The angle at which the tubes are viewed is defined by two horizontal slits such that the background is a strip of black velvet just below the ground glass diffusing screen. The scattered light from the precipitate is maximum, since the angle of scattering is small. Direct reflections are avoided by painting the slit and rack flat black (no linseed oil or varnish) and hiding the bottoms of the tubes below the second slit. This permits the tests to be read with very little eyestrain. Constant conditions are obtained by being entirely independent of daylight and by placing the

*From the Department of Agricultural Bacteriology, College of Agriculture, University of Wisconsin.

This work was supported in part by a grant from the Wisconsin Alumni Research Foundation and is published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

Received for publication, June 27, 1938.

tubes at some standard distance from the eye and from the light. A 60 watt bulb appears to provide sufficient illumination, although the box is wide enough (10 inches) to take a 200 watt lamp.

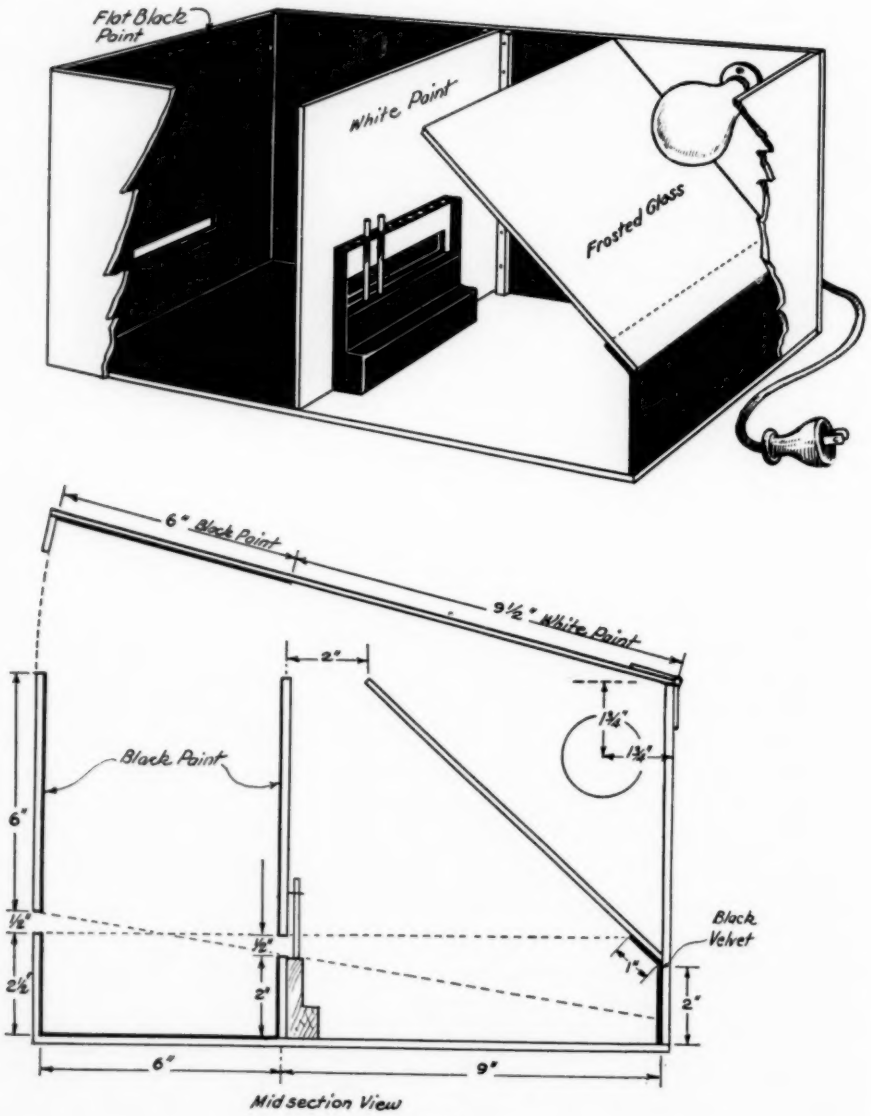


Fig. 1.

A smooth bed on which the tube rack could be slid into position from the side instead of lowering it from the top would improve the apparatus. Larger racks and partitions with slits enlarged downward would permit it to be used also for agglutination and hemolysin tests.

REFERENCE

1. Nickolls, L. C., and Thomas, John C.: An Improved Precipitation Viewing Apparatus, Brit. J. Exper. Path. 18: 55, 1937.

A NEW BLOOD CULTURE MEDIA APPARATUS*

PETER NARVAEZ, ELDRIDGE, CALIF.

THE success of making blood culture media is dependent directly upon the sterility that can be maintained during the transfer of blood to the media. Very often during this transfer the media become contaminated, since the blood must be added below 50° C. This article and diagram describe a new

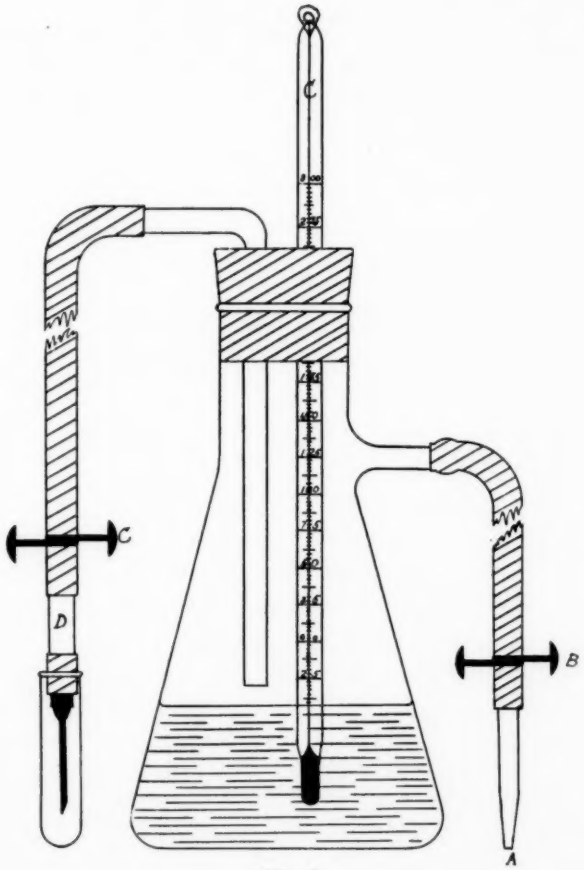


Fig. 1.

method which eliminates contamination to a great extent. It is inexpensive, easily constructed, and very well suited to a small laboratory. This method has been used in the laboratory of the Sonoma State Home for the past two years, with less than 1 per cent contaminations. The principle is here outlined.

*From the Bacteriological Laboratory, Sonoma State Home, Eldridge.
Received for publication, July 27, 1938.

Into a 1,000 c.c. suction flask, place 500 c.c. of bullion together with agar, stopper with a two-hole rubber stopper. In one hole place a 300° C. thermometer; in the second hole place a glass tube bent at right angles so that it reaches 1 inch above the media. The other end has a rubber tube 8 inches long fitted with a No. 9 gauge needle. Glass tube *D* can be placed somewhere near needle. Clamp rubber tube. Place a small test tube over needle to insure sterility at all times.

On the suction tip place a rubber tube 4 inches long, with a glass tube protruding and clamp in the middle. The entire apparatus, together with the media, is now ready to be autoclaved.

After the media have cooled to a proper degree, a Bunsen burner is held over glass tip *A*, and clamp *B* is opened to admit sterile air, after which the blood is to be added.

Remove test tube covering sterile needle and make vein or heart puncture. When clamp *C* is released, the blood enters the flask and is mixed with the media. When a suitable mixture is obtained, clamp as before and remove rubber tube from suction tip. From this tip, tubing or the pouring of plates can be accomplished very successfully with the media being sterile at all times. To insure greater sterility, an alcohol lamp can be held over glass tube *D* during the pouring of media.

A METHOD FOR THE DETERMINATION OF ERYTHROCYTE FRAGILITY, USING VAN ALLEN HEMATOCRIT TUBES FOR THE MEASUREMENT OF CHANGES IN VOLUME OF THE CELLS IN HYPOTONIC SALT SOLUTIONS*

GEORGE M. GUEST, M.D., AND MARY WING, M.S.
CINCINNATI, OHIO

THE procedure here described is designed to facilitate the measurement of swelling of erythrocytes in hypotonic salt solutions, making this measurement practicable in clinical studies of osmotic resistance or fragility of the red blood cells. The method is fairly rapid and requires only a small amount of blood.

Apparatus.—Either the original form of Van Allen (1925) hematocrit tube, or the modified form recently described (Guest, 1938), providing for automatic volume adjustment of the blood sample, can be used. In most instances from 12 to 16 tubes should be used, but this number can be increased or decreased to suit the needs of individual experiments. For centrifugation of the hematocrit tubes we have used the International centrifuges, Type SB, Nos. 1 and 2. For the original type of Van Allen tubes we have used for the most part the No. 1 centrifuge, with the 8-place head carrying Babcock test metal cups. The short Babcock test cups are fitted with wooden cylinders drilled perpendicularly with

*From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati.
Received for publication, August 2, 1938.

4 holes, each hole large enough to accommodate the stem of the Van Allen tube with its sealing clip. Alternatively, these Van Allen tubes can be carried in the No. 1 centrifuge in 10 ml. centrifuge tube holders with the 8-place head and 3-place trunnion carriers. For the modified Van Allen tube (longer and somewhat less sturdy) we use the 15 c.c. centrifuge tube holder, this holder being deep enough to support the hematocrit tube well above the bulb. These are placed in the No. 2 centrifuge with the 8-place head and 3-place trunnion carriers; thus, 24 tubes can be centrifuged at once if needed.

Solutions.—Salt solutions of different concentrations (indicated in Table I) are prepared by delivering from a burette different amounts of a 5 per cent stock solution of sodium chloride C.P. into 200 c.c. volumetric flasks. These are diluted to volume with distilled water and transferred to tightly stoppered Pyrex bottles. Such solutions, if kept in a refrigerator, will remain unchanged for several months. At the time of use, needed amounts of these solutions are transferred to a series of small vials into which the Van Allen tubes can be dipped for diluting the blood samples.

Blood Samples.—Either defibrinated or heparinized blood can be used, but oxalate, citrate, and fluoride anticoagulants should be avoided because of their effects upon the osmotic equilibrium of the cells. If the blood is drawn by vein puncture, both needle and syringe should be dry. Best results are obtained where the volume of cells in the sample is above 30 per cent. Where the volume of cells in a given blood is low, the sample should be centrifuged just sufficiently to throw down the cells, part of the plasma drawn off to bring the volume of cells well above 30 per cent, and the cells then resuspended in the remaining plasma—a glass bead being added to aid the mixing of the cells and plasma. In some instances it may be desirable to use a suspension of red blood cells, freed of their plasma, in 0.9 per cent sodium chloride solution (see Discussion). To obtain this, the blood sample is centrifuged, the plasma drawn off completely, and the cells are suspended in an approximately equal volume of the salt solution. One-half to 1 c.c. of either whole blood or such a cell suspension will easily suffice.

Procedure.—The manner of using both the original and the modified Van Allen hematocrit tubes is described more fully elsewhere (Guest, 1938). In using the original type of tube, blood is drawn into the stem up to the 100 mark. In using the modified tube, blood is drawn into the measuring stem until the capillary tube is completely filled. With either tube, the tip is then wiped and the blood drawn up slightly into the bulb, the appropriate salt solution is drawn into the bulb until it is nearly filled—leaving a small air bubble to facilitate mixing of the cell suspension—and the spring clip sealing device is fitted over the graduated stem. The dilution with 0.9 per cent sodium chloride in the first tube of the series should be made in duplicate or triplicate, since the cell volume determined in the first tube will be used as a basis for comparison of the values determined in the other tubes. For the subsequent dilutions, only single tubes need be used. The successive steps in the changing concentrations of the salt solutions used in the series, decreasing by 0.1 to 0.025 per cent, can be chosen to fit the needs of individual experiments. The decrements in the concentrations should be small throughout the range where hemolysis occurs, and in the tubes

just before the first trace of hemolysis becomes visible. This, of course, can be anticipated better when making repeat examinations of the blood of an individual subject. The filled tubes are left resting on their sides (a wire test tube rack turned on its side is convenient) for one to two hours at room temperature. They are then centrifuged until the cells are packed to constant volume. In the centrifuges designated above, a speed of 2,500 to 3,000 r.p.m. for fifteen minutes is easily sufficient.

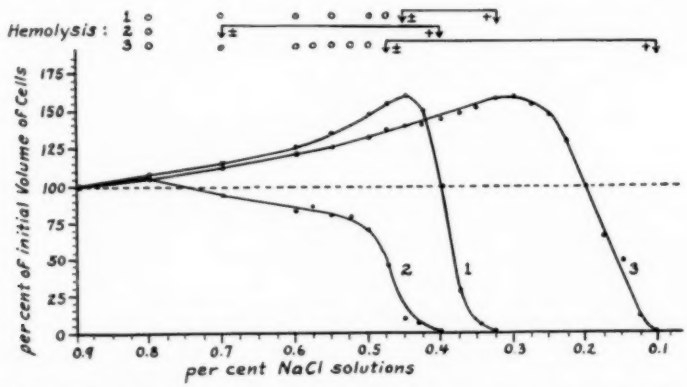


Fig. 1.—Hemolysis and changes in volume of red blood cells in hypotonic salt solutions. Observations on three conditions characterized by different fragilities of the red blood cells: *1*, normal (representing the values listed in Table I); *2*, familial hemolytic icterus; and *3*, erythroblastic anemia (Cooley's anemia).

Notation of Results.—After centrifugation, the degree of hemolysis (or its absence) in each tube is read from the color of the supernatant fluid in the bulb, and recorded by suitable symbols from zero, a trace, etc, to complete hemolysis, according to the usual practice in other methods for determining fragility of the red blood cells. The height of the column of packed cells in each tube is read from the graduations on the stem and recorded as shown in Table I. The values for the volume of cells in the several tubes are then divided by that of the first tube and multiplied by 100, in order to convert the readings to percentages of the volume of the cells in 0.9 per cent sodium chloride. For graphic representation, the latter values are plotted as illustrated in Fig. 1, and the degree of hemolysis at each point in the series is indicated by suitable symbols across the top of the chart.

DISCUSSION

The values listed in Table I, obtained according to procedure on a sample of normal human blood, illustrate the method of notation herewith described. Fig. 1 illustrates observations which have been made on samples of blood from subjects with conditions characterized by different fragilities of the red blood cells: *1*, normal (curve No. 1 represents the example given in Table I); *2*, familial hemolytic icterus; *3*, erythroblastic anemia (Cooley's anemia). The cells of the patient with hemolytic icterus showed the increased fragility that is characteristic of this condition, whereas the cells of the patient with erythroblastic anemia showed an increased resistance to hemolysis by the hypotonic salt solutions. Further studies of these and other types of bloods will be reported in later communications.

TABLE I

NOTATION OF RESULTS: HEPARINIZED NORMAL HUMAN BLOOD DILUTED IN VAN ALLEN HEMATOCRIT TUBES WITH SOLUTIONS OF SODIUM CHLORIDE OF DIFFERENT CONCENTRATIONS

TUBE NO.	Nacl PER CENT	VOLUME OF CELLS		HEMOLYSIS
		READINGS PER CENT	PERCENTAGE OF NO. 1	
1	0.900	39.6	100	0
2	0.800	42.3	107	0
3	0.700	45.3	114	0
4	0.600	49.4	125	0
5	0.550	53.1	134	0
6	0.500	58.7	148	0
7	0.475	61.2	155	0
8	0.450	63.9	161	±
9	0.425	59.3	150	+
10	0.400	38.8	98	++
11	0.375	11.2	28	+++
12	0.350	2.0	5	+++
13	0.325	0.0	0	Complete

The points on the curves represent the volumes of cells in the several salt solutions in percentage of their initial volume in 0.9 per cent sodium chloride solution. The curves first rise with swelling of all the cells up to the point where the first trace of hemolysis appears, and then fall when the loss of cells by hemolysis exceeds the increases in volume of the unhemolyzed cells. A generally accepted view of hemolysis is that the individual erythrocyte obeys the all-or-none law; i.e., when a cell is hemolyzed, all the hemoglobin escapes at once. Since the cells in any given sample do not all behave alike, the curves have variably rounded peaks and more or less gradual downward slopes. Thus, the shape of each curve indicates the relative tendencies of the cells in the given blood sample to disintegrate in salt solutions of different concentrations.

While this procedure is intended to serve most purposes of clinical study, details of the method can be changed easily to suit the needs of investigations of special problems concerning erythrocyte fragility. Solutions of other salts and salt mixtures, or of nonelectrolytes, can be used. Instead of the sodium chloride solutions of graded tonicities usually employed in cell fragility tests, Wiseman (1932) has recommended the use of hypotonic solutions prepared by diluting plasma, drawn from the cells to be tested with distilled water. The comparison of the fragility of cells in such hypotonic serum solutions with their fragility in simple salt solutions should be instructive, inasmuch as the blood plasma may contain substances which affect the osmotic resistance of the erythrocytes—either favoring or inhibiting hemolysis. With marked jaundice the color of the supernatant fluid may obscure the readings of the first traces of hemolysis. In such cases it may be advisable to get rid of the plasma in the manner described under preparation of the blood sample, and to work with a suspension of the cells in salt solution. It is to be noted, however, that the behavior of cells washed one or more times with salt solution may differ from that of unwashed cells.

SUMMARY

A method for determining red blood cell fragility in hypotonic sodium chloride solutions is described, wherein Van Allen hematocrit tubes are employed to measure changes in volume of the erythrocytes in each solution used in the

hemolytic series. The volumes attained by the cells in increasingly hypotonic solutions are expressed in percentage of their initial volume, and recorded graphically. Hemolysis also is read in the same tubes, and the points of beginning and complete hemolysis recorded according to usual practice.

REFERENCES

Guest, G. M.: Modified Van Allen Hematocrit Tube Providing for Automatic Volume Adjustment of the Blood Sample, J. LAB. & CLIN. MED. **24**: 75, 1938.

Van Allen, C. M.: An Hematocrit Method, J. LAB. & CLIN. MED. **10**: 1027, 1925.

Wiseman, B. K., and Bierbaum, O. S.: A New Method for Determining the Fragility of Red Blood Cells, Proc. Soc. Exper. Biol. & Med. **29**: 835, 1932.

Beebe, R. T., and Hanley, E. P.: A Method for Measuring the Fragility of Erythrocytes in Salt Solution, J. LAB. & CLIN. MED. **21**: 833, 1936.

Castle, W. B., and Daland, G. A.: Susceptibility of Mammalian Erythrocytes to Hemolysis With Hypotonic Solutions: A Function of Differences Between Discoidal Volume and Volume of a Sphere of Equal Surface, Arch. Int. Med. **60**: 949, 1937.

Daland, G. A., and Worthley, K.: The Resistance of Red Blood Cells to Hemolysis in Hypotonic Solutions of Sodium Chloride. Observations in Blood Disorders, J. LAB. & CLIN. MED. **20**: 1122, 1935.

Harvey, W. F.: Studies in Method and Standardization of Blood Examination. IV. Estimation of Erythrocyte Fragility and a Normal Standard, Edinburgh M. J. **44**: 100, 1937.

Lepeschkin, W. W.: Fragility of Red Corpuscles and Its Determination in Clinical Work, J. LAB. & CLIN. MED. **17**: 1250, 1932.

Ponder, E.: Mammalian Red Cell and the Properties of Hemolytic Systems, Berlin, 1934, Borntraeger.

Ponder, E., and Saslow, G.: The Measurement of Red Cell Volume. III. Alterations of Cell Volume in Extremely Hypotonic Solutions, J. Physiol. **73**: 267, 1931.

Saslow, G.: On the Supposed Partial Liberation of Haemoglobin From the Mammalian Erythrocyte, Quart J. Exper. Physiol. **19**: 329, 1929.

Warburg, E., and Winge, K.: A Method of Determining Osmotic Pressure of the Cations in the Serum and a Few Preliminary Results Obtained by It, Acta med. Scandinav Supp. No. 26, p. 500, 1928.

A MORE RAPID METHOD OF GUINEA PIG INOCULATION FOR THE DIAGNOSIS OF TUBERCULOSIS*

CARRIE I. WOOLSEY, M.A., CHICAGO, ILL.

FORMER methods of guinea pig inoculation for the diagnosis of tuberculosis have the following unsatisfactory factors: the length of time before a report may be expected; the possible death of the guinea pig due to the presence in the exudate of pathogenic bacteria other than tubercle bacilli; the possibility of the occurrence of a tuberculous guinea pig from contaminated surroundings.

Since results obtained by the intracutaneous injection of guinea pigs with tuberculous exudates recently reported by Herrold and Woolsey[1] tend to overcome these defects in the old procedures, the intracutaneous method has been adopted in the laboratory at Cook County Hospital and was used in the studies included in this report.

METHOD OF PROCEDURE

After shaving a small area on the side of the abdomen of a guinea pig, 0.4 c.c. of the infective material is injected intracutaneously. Within seven to twenty-one days, or in unusual cases, later, a nodule appears at the site of the injection, which averages about 2 mm. in diameter. The nodule is incised and smears are examined for the presence of acid-fast bacilli. Each guinea pig is observed every week and a notation made indicating its condition on each date. If two weeks pass between examinations, a closed nodule may become craterlike and consequently more easily and definitely proved positive. However, the earlier diagnosis would be missed by the delay. It should be noted, the early smear may demand a reasonable period of search to find the organisms. In an occasional case, the third or fourth examination of the pustule may be necessary before the organisms are found. As soon as the inguinal lymph nodes have formed on the side of the injection, the guinea pig may be killed, and an autopsy performed to verify the early report.

One guinea pig may be given more than one injection of the suspected fluid collected at various intervals but can be diagnosed and reported individually. In a similar manner two exudates from different sources of a given patient may be injected on opposite sides of a guinea pig, and each may be diagnosed and reported separately.

The following reports are illustrative of some of the advantages of the intracutaneous injection of guinea pigs.

A. G.—Spinal fluid of puncture December 14 reported positive in fourteen days.
Spinal fluid of puncture December 15 reported positive in twenty-one days.

*From the Department of Pathology and Bacteriology, Cook County Hospital, Chicago.
Received for publication, August 3, 1938.

Both processes developed on the same pig independently of each other. Regional adenopathy with deep hard nodules in the groin developed in three and five weeks, respectively. At five weeks the guinea pig was killed, and the autopsy revealed findings typical of tuberculosis.

The diagnosis had been reached in fourteen days.

D. S.—March 1, spinal fluid was received and cultured on Herrold egg yolk agar medium.[2, 3]

March 11, colonies of acid-fast bacilli were reported recovered from the culture.

March 11, a second spinal fluid was received and injected into a guinea pig. On the opposite side of the same pig, 0.4 c.c. of a suspension of the Herrold culture growth was injected.

March 22, a nodule produced by the suspension was reported positive for tubercle bacilli.

March 30, the second spinal fluid was reported positive after nineteen days.

Regional adenopathy developed on both sides, and the subsequent autopsy findings at five weeks were typical of tuberculosis.

In this way a culture may be readily verified.

J. W.—On January 20, specimens of spinal fluid and pleural fluid were injected on opposite sides of a guinea pig.

The spinal fluid was reported positive in twenty-three days, but the site of the pleural fluid injection remained negative throughout. Autopsy findings after three months revealed regional adenopathy only on the left side, where the spinal fluid had been injected, and generalized tuberculosis.

R. S.—Pleural fluid was reported positive in six days.

Pericardial fluid was reported positive in twenty days.

Autopsy at four weeks revealed regional adenopathy on both sides and generalized tuberculosis.

Urine from both kidneys of a patient has frequently been injected into opposite sides of a guinea pig to ascertain if one or both organs are involved.

Excepting sputa, secondary invaders in the exudates have not killed any pigs or interfered with the development of tuberculous lesions at the site of injection when given intracutaneously. A small abscess soon develops in such cases from which *Staphylococcus aureus,* hemolyzing streptococci, and *B. coli* among others have been isolated. Occasionally, acid-fast bacilli have been found even in this early abscess, but more often it heals over readily and is followed by the later nodule from which only the acid-fast bacilli are recovered.

Untreated sputa injected into the guinea pigs intracutaneously have been proved to contain tubercle bacilli in two weeks, although the direct examination of the sputum had failed to demonstrate them. Occasionally other bacteria in the sputum will kill the guinea pig, but if *B. fusiformis* is present in notable numbers, the anaerobe will burrow through the deep layers of the skin and eventually the guinea pig will have to be killed before the question of tuberculosis is settled.

Since the initial lesion is localized, a diagnosis may be made before or without an autopsy of the guinea pig. Such a localized initial lesion with a subsequent regional adenopathy is the proof of a positive injected animal.

In the total number of 173 guinea pigs included in this series, tubercle bacilli were recovered 72 times; or in 40 per cent of the exudates studied during

this period. They represent 27 different human exudates that were suspected of containing tubercle bacilli.

The average time for a positive diagnosis was twenty days, ranging from six to thirty-one days.

Twenty-five per cent of the positives were so reported within fourteen days.

Of the 173 guinea pigs of the series, 4 died of unknown cause, 1 on the sixth day, 2 on the eighth, and 1 on the twelfth day, respectively, and autopsies revealed nothing suggestive of tuberculosis. One was killed because of infection with *B. fusiformis.*

The first 36 guinea pigs reported negative were killed after six to eight weeks and autopsies were performed. They were in excellent condition and the autopsies revealed nothing suggestive of tuberculosis, therefore, the subsequent 60 negative guinea pigs of the series were kept as breeding stock since the injection had not retarded them in any way.

SUMMARY

The average time for the diagnosis of the tuberculous exudates by means of the intracutaneous injection of guinea pigs is three weeks in comparison with the six to eight weeks required by the old subcutaneous route.

Of the 173 guinea pigs injected in this series, only 5 died before the presence or absence of tubercle bacilli in the fluid could be determined.

Since the diagnosis is made from the initial lesion and substantiated by regional adenopathy and autopsy, there is little chance of a spontaneously infected guinea pig not being recognized as such.

Those guinea pigs that remain negative for six weeks may be kept as breeding stock.

REFERENCES

1. Herrold, R. D., and Woolsey, C. I.: Observations on Guinea-Pigs Given Intracutaneous Injections of Tuberculous Exudates, Arch. Path. **26**: 374, 1938.
2. Herrold, R. D.: Egg Yolk Agar Medium for the Growth of Tubercle Bacilli, J. Infect. Dis. **48**: 236, 1931.
3. Woolsey, C. I.: The Diagnosis of Tuberculosis. A Comparison of Results Obtained From Herrold Egg Yolk Agar Medium and With Inoculation of Guinea-Pigs, J. Infect. Dis. **49**: 177, 1931.

A SIMPLE METHOD FOR THE ASPIRATION OF BONE MARROW*

MAURICE MORRISON, M.D., AND A. A. SAMWICK, B.S., M.D.
BROOKLYN, N. Y.

THE study of the hematocytology of the bone marrow has become so necessary in the diagnosis of diseases of the hemopoietic system that a method for the aspiration of the marrow of the sternum must be simple, adequate, and devoid of danger. Furthermore, it must be one which allows repeated studies with the least amount of trauma.

Autopsy formed the only source of material for the study of the bone marrow until Ghedini (1908)[1] trephined and scraped the tibia of patients for diagnosis. Seyfarth (1922)[2] suggested the sternum as the site for biopsy since it was safe and the marrow was active. Modifications of the original technique have been presented,[3] but the customary use of the trephine prevented its universal application.

The examination of the bone marrow by mere aspiration of its contents, which was devised by Pianese as early as 1903,[4] and neglected because it did not supply material for the study of sections, was revived by Arinkin (1929).[5] With the slight modifications suggested by Sonnenfeld,[6] Arjeff,[7] Baserga,[8] and Reich[9] this method has since been widely used in many clinics.

The method that is presented here is as simple to do as lumbar- or venepuncture. It entails no greater psychic or physical trauma to the patient than either of these procedures. It consists of the use of a special steel needle,† 18 gauge, 2 inches in length, the point of which is so beveled that the left side is slightly higher than the right (Fig. 3). It is just as unnecessary to use local anesthesia, as it would be to resort to the latter for venepuncture. In more than 250 aspirations[10] we have had no instance in which the patient has been unduly annoyed by this procedure.

Procedure.—1. The instrument tray bearing the following is placed out of sight of the patient: (a) a 5 c.c. syringe (to draw up bone marrow); (b) an aspiration needle; (c) a hemostat; (d) iodine, alcohol, stick sponges, and sterile towels.

2. The region about the angle of Louis (sternomanubrial junction) is prepared with iodine and alcohol; the surrounding region is draped with 2 sterile towels and general asepsis is observed.

3. The point of entrance at the midpoint of the sternum just below the angle of Louis (Fig. 2) is then localized.

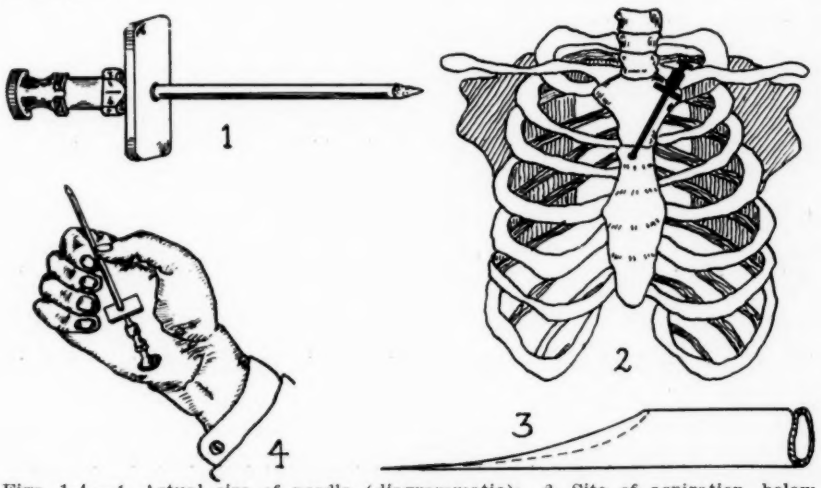
4. With the needle between the thumb and index finger in screwdriver fashion, at a point about one-half inch from the end, so that its fulcrum or

*From the Division of Hematology of the Departments of Medicine and Laboratories of the Jewish Hospital of Brooklyn.
Received for publication, August 12, 1938.
†Needle made by Becton, Dickinson and Co., No. 484 NT. (Fig. 1).

base rests snugly in the palm of the hand (Fig. 4), the skin is pierced almost perpendicularly and the periosteum is entered.

5. Downward pressure is exerted, turning the needle in screwdriver fashion.

6. In an elderly patient a sudden "give" is felt, indicating that the bone marrow cavity has been entered. In younger individuals the "give" is not readily apparent and one must desist from persistent pressure and work slowly and carefully, turning the needle from side to side, and aspirating occasionally.



Figs. 1-4.—*1.* Actual size of needle (diagrammatic). *2.* Site of aspiration, below angle of Ludwig. *3.* Sketch illustrating point of needle with bevel of left side higher than the right. *4.* Needle held firmly in the palm of the hand (screwdriver fashion).

7. The needle is *not* depressed at this stage to avoid breakage.

8. The obturator is removed, and the 5 c.c. syringe is attached. A minimum amount of bone marrow (usually 1 to 3 drops) is withdrawn, to avoid excessive dilution with peripheral blood.

9. On clean prepared slides thin smears of the marrow are made. At the same time smears of peripheral blood are prepared and all are stained by the usual methods. These may be studied with as much precision as ordinary blood smears.

Advantages of This Method.—1. It can be used in all cases because it minimizes mental and physical trauma.

2. It can be oft repeated.

3. The needle cannot break under ordinary circumstances, but if it should, it would only break at the junction of the base with the needle, and can easily be removed with a hemostat.

4. The higher left edge produces a "gouging of the bone," so that entrance into the bone is smooth.

5. The screwdriver motion avoids the "straight push" method which is fraught with danger and favors breaking of the needle.

6. When difficulty in obtaining post-mortem examinations is anticipated, bone marrow smears can be made in the preagonal state.

Disadvantages of Other Methods.—1. Usually other methods are too elaborate and cumbersome and frighten the patient.

2. Local anesthesia adds further risk with attendant discomfort. Sometimes the patient may have an idiosynerasy to novocain.

3. More involved procedures do not yield more information,[11] and, in addition written consent is usually required.

4. The "push method" involves great risk whether the needle is controlled by one or two hands.

Aspiration of the bone marrow is useless in the hands of one who is unable to identify the cells and interpret the findings.

REFERENCES

1. Ghedini, G.: Punctura esplorativa del middollo osseo, Clin. med. ital. **47**: 724, 1908.
2. Seyfarth, C.: Die Sternumtrepanation, Arch. f. Schiffs - u. Tropen-Hyg. **26**: 337, 1922.
3. (a) Morris, L. M., and Falconer, E. H.: Intravitam Bone Marrow Studies, Arch. Int. Med. **30**: 485, 1922.
   (b) Tuohy, E. O., and Gillespie, M. G.: A Trephine Modified to Secure Bone Marrow (Sternal) Biopsies, J. A. M. A. **104**: 404, 1935.
4. Pianese (1903), Quoted by Caronia, G.: Pediat. **30**: 607, 1922.
5. Arinkin, M.: Die Intravitale Untersuchungs Methodik des Knochenmarks, Folia haemat. **38**: 233, 1929.
6. Sonnenfeld, A.: Zur Technik der Sternalpunktion, Deutsche med. Wchnschr. **54**: 1380, 1928.
7. Arjeff, M. J.: Zur Methodik der Diagnostischen Punktion des Brustbeines, Folia haemat. **45**: 55, 1931.
8. Baserga, A.: La tecnica della sternopunctura, Riforma med. **50**: 1707, 1934.
9. Reich, O.: Modified Technic for Sternal Puncture and Its Value in Hematologic Diagnosis, J. Clin. & Lab. Med. **20**: 286, 1934.
10. Morrison, Maurice, and Samwick, A. A.: Clinico-Hematological Evaluation of Sternal Aspirations. Unpublished data.

A COMPARATIVE STUDY OF CERTAIN METHODS OF MEASURING BLOOD PRESSURE*

JOSEPH B. CADY, M.D., AND J. F. HERRICK, PH.D., ROCHESTER, MINN.

THE standard method of measuring the mean arterial blood pressure in the dog is by a direct communication between a cannulated blood vessel and a mercury manometer. The number of times that one can thus measure the blood pressure in the same intact dog is limited to the number of arteries available because the cannulated artery has to be ligated at the end of each experiment. Another important objection to the standard method is that true pulse pressures cannot be obtained. The auscultatory method has been used by several investigators for observing pulse pressures. In our experience, however, this auscultatory method has not been entirely satisfactory. The systolic pressures are always lower and the diastolic pressures definitely higher than those obtained by other methods. If one is interested in differences of pressure only, the auscultatory method may be adequate.

The "hypodermic" manometer developed by Hamilton, Brewer, and Brotman has proved to be a most satisfactory method for measuring blood pressure. It has been used in this laboratory for three years. To those inexperienced with the method, however, the values appear too high, and it was because of this criticism that these comparative studies of the reliability of the instrument were made preparatory to certain studies of differential pressure, which will be reported later. We have endeavored to check the hypodermic manometer against the mercury manometer: (1) by comparison with mean pressures obtained by the standard method, using a hypodermic needle instead of a cannula (the hypodermic needle offers the necessary constriction for damping the oscillations, thus permitting registration of the true mean blood pressure), and (2) by comparison with systolic and diastolic pressures obtained by auscultation.

The main portion of our investigation has been concerned not so much with absolute basal pressures as with the difference in pressure between the carotid and femoral arteries. The present study is, therefore, not intended to present values for the "normal" blood pressure of dogs, but only to check the reliability of the apparatus. We did not take the precautions which others have taken to insure the lowest possible pressures, such as maintaining absolute quiet and a soft bed for the animals, withholding food and administering morphine. For our purposes all that was essential was that the animals be sufficiently trained to lie quietly on the table and submit without struggling to direct arterial puncture. We have found that morphine alone will often

*From the Mayo Foundation, Rochester, Minn.
Received for publication, August 15, 1938.

reduce the femoral blood pressure from 10 to 15 per cent. In addition we have found that, by more strict adherence to the aforementioned precautions, we can obtain figures with the Hamilton manometer which approach those published by other workers.

The Hamilton optical manometer has been devised to record blood pressure by direct puncture of the arteries of trained, unanesthetized animals. It has been constructed to conform to the requirements for accurate measurement set forth by physicists and physiologists, namely: the possession of a membrane of sufficient sensitivity and high frequency, in a system which is otherwise practically nonexpansile. In our experience, under the conditions mentioned, this manometer has recorded figures which are consistently and considerably higher than those which have been quoted in the literature. Thus, in the case of one dog whose femoral blood pressure has been recorded fifteen times during the past year, the systolic and diastolic variations have been 170 to 215 and 86 to 114 mm. of mercury, respectively (average 196/99). The average of 83 observations on 24 dogs whose blood pressure was similarly recorded was 201 mm. systolic and 107 mm. diastolic. We would like to reemphasize the fact, however, that these figures are not to be taken as basal femoral pressures.

METHODS

To compare the mean pressures a 20 gauge needle was inserted into one of the femoral arteries of an unanesthetized dog and connected by rubber tubing to the usual type of U-tube mercury manometer. At the same time a second 20 gauge needle, connected by lead tubing with the Hamilton manometer, was inserted into the contralateral femoral artery. A series of several readings of the mean pressure was made and recorded from the mercury manometer at the same time that a series of pulse pressure tracings was photographically recorded from the optical manometer. The mean pressures were calculated from the latter tracings by graphic integration of the pulse pressure curves with a planimeter, since it has been repeatedly demonstrated that the mean pressure is the integrated rather than the arithmetic mean of the systolic and diastolic pressures. In some series of observations, variations in pressure were produced by injections of histamine intravenously.

Further observations were made to compare the direct and indirect methods of recording systolic and diastolic blood pressure. For this purpose a pneumatic cuff was constructed (after the manner described by Freeman and Page) which was 5 by 14 cm. in size and incased in a cloth cuff 35 cm. long. This cuff was wrapped snugly about the animal's leg in such a manner that its lower border covered a small portion of a 2 cm. Bowles type stethoscope chest piece which was attached to the shaven skin over the posterior tibial artery above the ankle by means of an adhesive paste. The systolic and diastolic pressures were read and recorded from a mercury Baumanometer at the same time an assistant photographically recorded the femoral pulse pressure by a Hamilton manometer connected with the femoral artery of the opposite leg. A series of such simultaneous observations (about thirty seconds apart) was made on each animal.

TABLE I

BILATERAL ARTERIAL BLOOD PRESSURES RECORDED SIMULTANEOUSLY WITH THE HAMILTON OPTICAL MANOMETER

ARTERIES	OBSERVATION	SYSTOLIC (MM. HG)		DIASTOLIC (MM. HG)	
		RIGHT	LEFT	RIGHT	LEFT
Femoral	1	227	226	108	111
	2	233	234	112	115
Femoral	1	204	202	106	103
	2	209	207	108	105
Femoral	1	193	189	103	99
Femoral	1	198	198	122	125
Femoral	1	185	186	88	90
Carotid	1	176	179	125	126
	2	171	175	121	123
	3	173	178	127	129
Brachial	1	140	138	109	108
	2	142	146	115	114

RESULTS

To demonstrate the sphygmic equality of the corresponding vessels on the two sides of the dog, observations were simultaneously recorded as shown in Table I. From these data we feel safe in assuming that, under normal conditions, the two femoral arteries have the same blood pressure. A comparison of the mean pressures obtained by the methods described is recorded in Table II. In our series of observations the greatest difference between any two simultaneously recorded figures was 10 mm. of mercury, and the average difference was 2 mm.; there was no apparent consistency about which method gave the higher values. Had the mean pressures been determined in the usual manner, that is, by taking half the sum of the systolic and diastolic values, the average for the series would have been 153 mm. of mercury, 30 per cent higher than the figure obtained with the mercury manometer.

TABLE II

A COMPARISON OF OBSERVATIONS OF MEAN BLOOD PRESSURE AS DETERMINED WITH THE HAMILTON OPTICAL MANOMETER AND THE MERCURY MANOMETER

OBSERVATION	MEAN PRESSURE (MM. HG)	
	HAMILTON MANOMETER	MERCURY MANOMETER
1*	125	126
2*	79	74
3*	122	114
4*	120	130
5*	101	96
1	127	123
2	120	125
3	125	127
4	125	125
1	128	125
2	133	123
3	124	121
4	126	117
1	123	117
1	137	134
1	120	114
2	112	116
3	122	120
4	120	117
Mean	120	118

*Changes in pressure brought about by the intravenous injection of histamine.

Table III shows a comparison of the systolic and diastolic pressures recorded optically from the femoral artery and simultaneously observed by auscultation over the contralateral posterior tibial artery. We have so far found it impossible to obtain readings by the auscultatory method that will compare closely with those recorded by the hypodermic manometer. Using the optical instrument on human subjects, Hamilton and his associates found that the systolic values were 3 to 4 mm. higher and the diastolic about 9 mm. lower than the figures obtained simultaneously by the usual clinical method. By the methods which we have used on dogs, we have, in most instances, been unable to find a correlation as close as this. This has been true whether we used the dorsalis pedis artery (as recommended by Freeman and Page) or the posterior tibial artery, and regardless of the width of the cuff or the size of the stethoscope bell. Our closest figures have been secured when larger dogs with relatively larger vessels were used and when the posterior tibial artery above the ankle was employed.

TABLE III

A COMPARISON OF THE INDIRECT (AUSCULTATORY) AND DIRECT (HAMILTON MANOMETER) METHODS OF ESTIMATING ARTERIAL BLOOD PRESSURE

OBSERVATION	SYSTOLIC (MM. HG)		DIASTOLIC (MM. HG)	
	DIRECT	INDIRECT	DIRECT	INDIRECT
1	182	174	100	108
2	187	174	104	110
3	179	178	102	108
1	225	214	115	124
2	222	212	114	120
1	223	220	96	120
2	230	226	102	112
3	220	216	95	116
4	210	202	101	114
5	215	210	102	110
1	206	166	96	92
2	202	164	86	88
3	201	158	90	96
4	205	166	90	88
1	220	194	97	118
2	210	196	93	124
3	206	190	99	120

The generally accepted clinical procedure in ascertaining the diastolic blood pressure is to note the point in the descent of the column of mercury at which the tone of the sounds changes and begins to fade—the fading of the so-called fourth phase. This was found to give values considerably higher than those recorded optically, and it was discovered that a much closer diastolic correlation resulted when the point of total disappearance of the sounds was used (see the next to the last series of observations in Table III).

It is possible that our figures may show a closer correlation than is apparent from the table. The systolic pressure is known to vary at different levels of the vascular tree. Hamilton and his associates found the pressure in the dorsalis pedis artery to exceed that in the femoral artery in man by 25 mm. of mercury. On the other hand, Dawson found that the pressure in the saphenous artery in 2 dogs to be 30 per cent lower than that in the femoral artery. We have recorded the pressure simultaneously in the femoral and

opposite posterior tibial arteries by the Hamilton method in 3 dogs and found the femoral pressure to be definitely higher (10 to 15 mm.). From such a small number of observations it is obviously impossible to draw conclusions. However, the data of previous workers and ourselves indicate that one may not be justified in assuming that the systolic pressure in the posterior tibial artery is the same (within the limits of experimental error) as that in the femoral artery.

An observation which we believe to be significant and which was made repeatedly showed that the act of inserting a needle for direct measurements of arterial pressure does not apparently increase the arterial pressure in the trained unanesthetized dog. This observation has been made by taking control auscultatory readings before the needle was inserted into the artery of the opposite leg, with the almost constant finding that subsequent readings are not appreciably elevated above the control readings.

SUMMARY AND CONCLUSIONS

A sphygmic equality exists between the two femoral arteries of the dog.

There is a satisfactory correlation between the integrated mean blood pressure, recorded with the Hamilton hypodermic manometer, and the mean pressure, read from a standard mercury manometer.

The values for the systolic and diastolic blood pressures observed over the posterior tibial artery by the auscultatory method did not agree with those for the opposite femoral artery recorded by the hypodermic manometer.

REFERENCES

1. Hamilton, W. F., Brewer, George, and Brotman, Irving: Pressure Pulse Contours in the Intact Animal. I. Analytical Description of a New High-Frequency Hypodermic Manometer With Illustrative Curves of Simultaneous Arterial and Intracardiac Pressures, Am. J. Physiol. **107**: 427, 1934.
2. Freeman, N. E., and Page, I. H.: Hypertension Produced by Constriction of the Renal Artery in Sympathectomized Dogs, Am. Heart J. **14**: 405, 1937.
3. Hamilton, W. F., Woodbury, R. A., and Harper, H. T., Jr.: Physiologic Relationships Between Intrathoracic, Intraspinal and Arterial Pressures, J. A. M. A. **107**: 853, 1936.
4. Dawson, P. M.: The Lateral Blood "Pressures" at Different Points of the Arterial Tree, Am. J. Physiol. **15**: 244, 1906.

ROSE BENGAL EXCRETION AND HIPPURIC ACID SYNTHESIS TESTS OF LIVER FUNCTION: A COMPARISON*

W. P. Stowe, M.D., San Francisco, Calif.

IN RECENT years numerous tests of liver function have been devised and advocated. In the short series of proved cases here presented several of these tests have been tried. In none of them did the galactose tolerance test prove positive, nor were icteric index and bilirubin findings parallel to the severity of the liver damage as shown at operation or autopsy. Serum Takata-Ara findings became positive at serum albumin-globulin ratios of 2:1 or less, as did also increased average diameters of the red blood cells above 6.9 micra, by the halometer method applied to dried smears.

Our present interest is, however, centered around two of the simplest and apparently most accurate of the liver function tests.

The rose bengal excretion test is that reported by Delprat and Stowe.[1, 2] Details may be obtained from articles quoted, or from the eighth edition of Todd and Sanford.[3] Photelometric methods of reading the test are also available in publications of Giordano and Eager[4] and of Stowe and Delprat.[5] Both techniques are completely described in the recent revision of *Approved Laboratory Technic* by Kolmer.[6] Briefly, the test is carried out by injecting intravenously 10 c.c. of 1 per cent solution of rose bengal in saline; waiting two minutes for blood stream mixing, and removing and oxalating a sample of blood. Six minutes later a second blood sample is removed and oxalated. Colorimeter comparison of plasma from the two samples shows a 50 per cent decrease of rose bengal in the second as compared to the first, providing the liver is normal. Greater degrees of retention in the blood stream are proportional to decreased liver function.

The hippuric acid synthesis test of Quick[7] is carried out by feeding the patient 6 gm. of sodium benzoate with a light breakfast. The entire next four hours' urine is saved, acidified slightly with acetic acid, evaporated to 100 c.c. if necessary, and, when cool, further acidified to Congo red with full strength hydrochloric acid. After this it is stirred vigorously until precipitation of hippuric acid occurs, and allowed to stand several hours; the crystals are then filtered off through a Büchner funnel, dried, and weighed. To the weight of the crystals thus obtained is added 0.33 gm. per 100 c.c. of filtrate, for hippuric acid still dissolved in this filtrate. The total figure is then multiplied by 0.68 to convert to benzoic acid in terms of which it is reported. A four-hour excretion of 2.5 gm. to 3.5 gm. as benzoic acid indicates normal

*From the St. Luke's Hospital, San Francisco.
Received for publication, August 22, 1938.

liver synthesis. Reduction below 2.5 gm., in the presence of relatively normal kidney function, is evidence of liver damage.

Seven cases are presented in Table I. All diagnoses, except that for patient 1, were proved at operation or autopsy. The early slight biliary cirrhosis in patient 7 was shown by biopsy at operation subsequent to the last rose bengal reported. The diagnosis in patient 1 was based on history, positive Wassermann findings, prompt response to treatment, and a later follow-up of apparently perfect health after a year's treatment. In the table the rose bengal function is reported in percentage of normal liver function; in column two, hippuric acid excreted in four hours, as grams of benzoic. In column three, for purposes of comparison with the rose bengal, hippuric acid excretion is figured as percentage of normal, using 2.5 gm. as normal output. In all except patient 7 the similarity of findings of these two simple tests is obvious. In this last patient, in view of the quite rapid return of rose bengal to normal under glucose therapy, the hippuric acid test proved the more accurate from the standpoint of permanent liver cell damage.

TABLE I

PATIENT	PROVED DIAGNOSIS	ROSE BENGAL FUNCTION	HIPPURIC ACID EXCRETION	HIPPURIC ACID FUNCTION
		Per cent	*Gm.*	*Per cent*
1. Mrs. A.	Syphilitic cirrhosis	34	0.9	35
	After 2 mo. treatment	77	1.8	72
2. Mr. U.	Carcinoma of head of pancreas	90	2.8	110
3. Mr. V.	Subacute hepatitis Nitrobenzol poisoning	35	0.75	30
4. Mrs. P.	"Cardiac cirrhosis" rheumatic heart, 14 yr. decompensated	55	1.1	44
5. Mrs. D.	Advanced cirrhosis	25	0.3	12
6. Mrs. I.	Advanced cirrhosis	20	0.43	17
7. Mrs. C.	Stone in common duct, slight biliary cirrhosis	30	2.05	82
	After one month intravenous glucose	90	Not repeated	---

SUMMARY AND COMMENT

Liver function values, as determined by two simple tests, are compared in 7 patients with proved diagnoses. One (rose bengal) is a purely secretory test; the other (Quick's hippuric acid synthesis) is a metabolic test. A striking parallelism of these tests to each other and to the liver condition is shown. In view of the close agreement between the two tests it is suggested that the rose bengal be used where speed is of importance (one hour to complete, as compared to twelve hours or more for hippuric acid test). Also it should be used where kidney function is known to be impaired, since this impairment vitiates the results in Quick's test. Where severe jaundice is present and in laboratories where apparatus and reagents are limited, the test of Quick is technically the more feasible and the one to be recommended.

REFERENCES

1. Delprat, G. D., and Stowe, W. P.: The Rose Bengal Test for Liver Function, J. LAB. & CLIN. MED. **16**: 923, 1931.

2. Stowe, W. P., Delprat, G. D., and Weeks, Alanson: The Rose Bengal Test of Liver Function, Am. J. Clin. Path. 3: 55, 1933.
3. Todd and Sanford: Clinical Diagnosis by Laboratory Methods, ed. 8, Philadelphia, 1936, W. B. Saunders & Co., p. 391.
4. Giordano, A. S., and Eager, Dean: The Rose Bengal Liver Function Test as Adapted to the Sheard-Sanford Photelometer, Am. J. Clin. Path. 5: 417, 1935.
5. Stowe, W. P., and Delprat, G. D.: The Rose Bengal Test of Liver Function: Photelometric Method, J. Lab. & Clin. Med. 20: 1297, 1935.
6. Kolmer, J. A., and Boerner, F.: Approved Laboratory Technic, ed. 2, New York, 1938, D. Appleton-Century Co., Inc., p. 237.
7. Quick, A. J.: The Synthesis of Hippuric Acid: A New Test of Liver Function, Am. J. M. Sc. 185: 630, 1933.

A SIMPLE METHOD OF OBTAINING SPUTUM FOR PNEUMOCOCCUS TYPING*

Jack Rom, M.D., Detroit, Mich.

IN INFANTS and young children and in acutely ill pneumonia patients who do not expectorate, it is frequently difficult or impossible to obtain sputum for pneumococcus typing. The method of culturing direct pharyngeal and laryngeal swabs is frequently unsatisfactory, while swabs by laryngoscopy and lung suction by means of transthoracic pulmonary puncture are methods not applicable to general usage. For this reason we have employed a method of obtaining material for pneumococcus typing, which we believe is simple, accurate, and practical for use both in the hospital and in the home. It includes the following: 20 to 50 c.c. Luer syringe (50 c.c. preferable); soft rubber catheter (size dependent upon age of patient); small sputum bottle containing 4 to 5 c.c. of sterile saline; and small amount of sterile glycerin.

The catheter is connected to the syringe, a small amount of glycerin is placed on its tip, and the catheter is then passed through the nares back to the nasopharynx and thence downward to the oropharynx. It is then passed slowly down to the larynx. When the catheter reaches the larynx, the patient frequently coughs. The material in the vicinity of the larynx and lower pharynx is then aspirated into the syringe by means of repeated suction. If insufficient material is obtained, the catheter is withdrawn slightly and the aspiration is repeated. After sufficient material has been secured, the catheter is withdrawn, the sterile saline is drawn up into the syringe, and the material flushed out into the sputum bottle.

This method was employed in 72 cases of pneumonia in which sputum was not expectorated. It was possible by this method to obtain sufficient material for bacteriologic study in all of the cases. The material obtained

*From the Department of Medicine of Wayne University and the Detroit Receiving Hospital.
Received for publication, August 22, 1938.

was examined both by the direct Neufeld method and by mouse injection. The results in these cases are shown in Table I.

TABLE I

CASES	PNEUMOCOCCUS FOUND AND TYPED		NO PNEUMOCOCCUS FOUND		TOTAL
	DIRECT	MOUSE	NO OTHER ORGANISMS	OTHER ORGANISMS	
Adults	16	25	1	3	29
Children	20	38	4	1	43
Total	36	63	5	4	72
	63				

SUMMARY

A method of aspirating sputum for pneumococcus typing is described. The results obtained in 72 cases of pneumonia indicate that this method is applicable in those cases in which sputum is not expectorated.

A SIMPLE DEVICE FOR CHANGING THE LIGHT INTENSITY IN THE X-RAY ILLUMINATOR*

EDWARD J. VAN LIERE, PH.D., M.D., AND DAVID W. NORTHUP, PH.D.,
MORGANTOWN, W. VA.

TWO difficulties are encountered in using an ordinary x-ray illuminator or view box in tracing teleoroentgenograms. First, the heat generated by the electric light bulb is apt to injure the film; often too, the film becomes so warm that the hand which is used for tracing cannot be allowed to rest on the film. Second, it is not easy, if not impossible, to regulate the intensity of light transmitted through the film.

Both of these difficulties can be obviated by the use of a simple and inexpensive device. A foot rheostat, such as is used in operating an electric sewing machine and as shown in Fig. 1, can be inserted in series with the electric light bulb in the x-ray illuminator. Simply by pressing down the foot a wide range of light intensity is instantly available without the necessity of using the hands, so that the latter may be used for tracing the x-ray silhouette. Further, if the film becomes too warm, the amount of light can be cut down so that the heat will not injure the film or become too hot for the hand. The electric light goes out automatically when the foot is released, so that the light cannot be accidentally left on and the film ruined.

In making the suggestion that a rheostat be used to vary the intensity of the light transmitted, the authors appreciate that most of the modern view boxes are equipped with mercury vapor lamps. It is obvious that the rheostat

*From the Department of Physiology, West Virginia University, Morgantown.
Received for publication, August 4, 1938.

could not be used in this type of view box; there are, however, still many view boxes in use which are not so equipped. A criticism which might be raised is that dimming a filament bulb may add an element of red to the transmitted light; many roentgenologists object to this. It is felt that this latter criticism is probably of no great importance if the rheostat is used while tracing cardiac silhouettes.

Fig. 1.

The authors feel then that such a device as described is especially useful in tracing the outline of the heart. The change in the intensity of the light often is of distinct help in tracing the apex, and the ability to be able to control the amount of heat generated makes it possible to work leisurely and carefully. Not only, however, is the device useful in tracing teleoroentgenograms, but it is also of use in studying the ordinary x-ray film where it is desirable to change the light intensity.

A MANUAL OF NEUROHISTOLOGIC TECHNIQUE

OSCAR A. TURNER, M.D., NEW HAVEN, CONN.

(Continued from the April issue, page 748.)

CHAPTER V

STAINING OF FAT

Some form of lipoid stain is generally required in any routine study of the nervous system. These stains are easy to do and give satisfactory results, although there is a tendency for all of them to fade in time. Of necessity, all are done on frozen sections and all are mounted in a medium containing no fat solvent.

Sudan III.—This is a commonly employed stain which is applicable to all types of tissue.

1. Frozen sections at 20 microns of formalin-fixed tissue are washed thoroughly in distilled water. Tissue embedded in gelatin may be used.
2. Transfer sections to 70 per cent alcohol and allow to remain for one minute.
3. Stain in a saturated solution of sudan III in 70 per cent alcohol for about twenty minutes.
4. Rinse in 70 per cent alcohol.
5. Counterstain in hematoxylin.
6. Wash well in two changes of distilled water.
7. Draw sections on a clean glass slide and mount in gelatin, Arabic sugar or glycerin jelly.

The droplets of fat are colored orange or orange red and stand out fairly well from the light orange background. The cell nuclei are stained, the depth of staining depending upon the type of hematoxylin used and the time of staining in it. Weigert's hematoxylin is a satisfactory stain for the nuclei and Delafield's may be used also. Mayer's hematoxylin gives an excellent nuclear stain. However, if the tissue has been embedded in gelatin before sectioning, it is necessary to use Ehrlich's hematoxylin for the nuclear stain. The preparations are best stored in the dark.

Herxheimer's Fat Stain (Sudan IV, Scharlach R, Scarlet Red).—This method, utilizing a derivative of sudan III as a dye, has become widely used and gives results which are superior to the older sudan III method.

1. Frozen sections of formalin-fixed tissue are cut at about 20 microns.

2. The sections are placed in distilled water.

3. Transfer to the fat stain made as follows:

Place an excess (1 to 2 gm.) of scarlet red dye in a wide-mouthed bottle. To this add the following mixture:

Absolute alcohol	70 c.c.
10% sodium hydroxide	20 c.c.
Distilled water	10 c.c.

Heat and place in the incubator at 37° C. for one hour. Cool in running water, and filter through three thicknesses of coarse filter paper into a large watch glass crystal. Allow solution to stand for twelve hours before using. It should not be more than five to six days old when used.

4. Cover watch glass with a glass plate and heat until the glass plate is sealed with the steam which arises. Allow to stand twenty minutes.

5. Wash in two changes of distilled water, allowing the sections to remain in the last wash for about three hours.

6. Shake in Mayer's hematoxylin diluted with water to about 50 per cent of its original strength. Allow to remain overnight, if necessary.

7. Wash in distilled water and then in tap water for two minutes.

8. Draw sections on clean glass slides under water, dry with filter paper and mount in glycerin jelly.

The fat is seen as small and large globules which are stained a scarlet red. The nuclei stain blue and are more distinct than in the sudan III method. The method has the disadvantage of taking more time to complete, but the results are correspondingly better. However, Hassin recommends placing the sections in about 20 c.c. of water to which 5 or 10 drops of Ehrlich's hematoxylin have been added, and allowing them to remain only fifteen minutes. The Ehrlich's hematoxylin is necessary if the tissue has been embedded in gelatin before sectioning. Otherwise, any hematoxylin may be used. The criticism has sometimes been raised that heating the sections tends to disperse or displace the fat globules. This will not take place if the heating is not carried to an excess. Filtering the staining solution before use helps prevent the formation of precipitates. The solution should always be kept covered to prevent evaporation.

Nile Blue Sulfate Stain.—Occasionally one is interested in obtaining an insight into the nature of the lipoidal substances contained in the cells which take the fat stain. Although it is obvious that no one single method is specific for any one type of lipoid and that a single fat globule may contain several different lipoid substances, the method of Lorrain Smith has proved of value in histochemical investigations. The method is as follows:

1. Frozen sections of formalin-fixed tissue are washed thoroughly in distilled water.

2. Sections are placed for ten minutes in a saturated aqueous solution of Nile blue sulfate.

3. Wash for two to three minutes in distilled water.
4. Mount in glycerin jelly.

Fatty acids are stained blue while neutral fats take a pink to red color. Mixtures of these two substances are purple or some shade of purple, depending upon whether the neutral fats or fatty acids predominate. Other lipoid bodies, such as myelin and lipochrome pigments, assume a blue color. Nuclei are stained dark blue and protoplasm a light blue. The stain is not permanent and fades in two to three days. Differentiation may be carried out in 1 per cent acetic acid if the staining is too deep.

Chapter VI

Nonmetallic Glia Stains

With the recent additions to our knowledge of the interstitial cells of the nervous system, many new selective staining methods have been devised to demonstrate these cells. The division here into metallic and nonmetallic methods has been made solely for convenience. Certain methods lend themselves more readily to tumor tissue and others more to the study of the degenerative and inflammatory diseases. The special applications of each will be noted in the descriptions of the individual stains.

Mallory's Phosphotungstic Acid-Hematoxylin Stain.—The best results with this method are obtained on paraffin sections of Zenker-fixed tissue. However, good results may also be had from the use of formalin-fixed material which has been treated with Zenker's fluid, or passed through the Weigert mordants as described by Kernohan. The stain is one of the simplest and most useful of the nonmetallic methods for the study of glial tissue in tumors.

1. Paraffin sections of Zenker-fixed tissue are brought to water and treated with Gram's iodine and hypo as usual to remove the mercuric chloride crystals.
2. After washing thoroughly, transfer sections to 0.25 per cent potassium permanganate solution and allow to remain for from twenty to thirty minutes.
3. Wash well in water and treat in 5 per cent oxalic acid solution until the sections are bleached white. They should remain here at least ten minutes.
4. Wash thoroughly in several changes of distilled water.
5. Stain in Mallory's phosphotungstic acid-hematoxylin solution for twenty-four to forty-eight hours. The stain is prepared as follows:

Hematein ammonium	0.1 gm.
Phosphotungstic acid crystals	2.0 gm.
Water	100.0 c.c.

Dissolve the hematein ammonium in a little water with the aid of heat and when cool add the remaining ingredients. Ripen by the addition of 0.1 c.c. of hydrogen peroxide or allow to ripen spontaneously for several months.

6. Rinse in water.

7. Dehydrate in 95 per cent alcohol, absolute alcohol, and clear in xylol. Mount in balsam.

Fibrin, fibroglia, neuroglia, and myoglia fibrils are stained blue while the intercellular connective tissue fibrils are stained a pale reddish brown or remain colorless. Collagen tends to take a reddish-pink color in most preparations. The coarse elastic fibers sometimes assume a pale purplish-blue color. If after step 5 the sections are placed in a 10 to 20 per cent alcoholic solution of ferric chloride for one to three minutes followed by thorough washing in water, the intercellular connective tissue fibers are completely decolorized, and the blue-stained glial fibers assume a somewhat deeper hue. The cell nuclei are blue, and the cytoplasm has a pale pink or brownish-pink color. Blepharoplasts may occasionally be stained a pale blue color.

It is essential that the stain be well ripened. The best results are obtained when hematein ammonium is used and allowed to ripen spontaneously for six months to a year. Stain which has been allowed to stand for a year or more gives an intense color to the tissues. Hematoxylin may be used instead of hematein ammonium, but requires twice the amount of hydrogen peroxide to cause ripening.

Formalin-fixed tissue may be employed. For this, Kernohan recommends that the unembedded blocks of tissue be treated four days in Weigert's primary mordant, followed by two days in Weigert's secondary mordant, after which the tissue is embedded in paraffin, sectioned and stained as usual. The same method may be employed on tissue already sectioned and mounted on glass slides. The deparaffinized sections remain in the primary mordant ten to twelve hours, and in the secondary mordant five to six hours. Care should be taken that the sections do not float off the glass slides. Fairly good results may be obtained also if small blocks of the tissue, which has been fixed in formalin or deparaffinized sections, are allowed to remain for three days in Zenker's fixative or 5 per cent potassium bichromate. If Zenker's fixative is used, the sections must be passed through iodine solution and 5 per cent hypo to remove the mercuric chloride crystals. Origanum and other oils cause the stain to fade. When mounted in balsam and not exposed unduly to direct sunlight, the preparations are reasonably permanent.

This method finds its greatest use in the study of tumors, especially the gliomas. It should be noted that the method is not selective for neuroglia, but also stains fibroglia and myoglia as well. The degree of impregnation of the elastic fibers is usually not sufficient to cause difficulty in interpretation.

Bailey's Ethyl Violet-Orange G Stain for Fibrillary Neuroglia.—This method was originally intended for use on Zenker-fixed tissue, but Davidoff has described a preliminary treatment whereby good preparations can be obtained on formalin-fixed material. Sections of paraffin-embedded tissue are placed in 50 c.c. of water to which 10 drops of ammonia have been added and allowed to remain for twenty-four hours at room temperature. The ammonia is then washed out with a slow stream of running water for one to two hours, and the sections are treated in Zenker's fluid for twelve to twenty-four hours.

They are then passed through Gram's iodine and hypo to remove the crystals of mercuric chloride and the stain carried out as with tissue fixed originally in Zenker's solution. The method is as follows:

1. Place for three days in a 3 per cent solution of potassium bichromate.

2. Rinse in water and transfer sections into a solution of ethyl violet-orange G and allow to remain for twelve hours. The staining solution is prepared as follows:

Ethyl violet	1.0 gm.
Orange G	0.5 gm.

The dyes should be weighed accurately. Add 100 c.c. of distilled water and stir thoroughly. Place in the incubator at 37° C. for twelve to twenty-four hours for precipitation to occur. Decant the supernatant fluid, and wash the precipitate several times with distilled water. Place in the incubator to dry. Make a saturated solution of the dried precipitate in absolute alcohol. Use this as a stock solution. For staining, use 1 part of the stock solution to 3 parts of 20% alcohol.

3. Blot the sections and agitate quickly in anhydrous acetone.

4. Place in toluol for two to three seconds.

5. Flood the slide with pure oil of cloves.

6. Differentiate in a mixture of 3 parts of oil of cloves and 1 part of 96 per cent alcohol.

7. Rinse in pure oil of cloves.

8. Pass sections through several changes of toluol and clear in several changes of xylol. Mount in balsam.

The neuroglia fibers appear dark violet against an orange background. The nuclei, fibrin, and erythrocytes are also stained. There is sharp staining of the myoglia and fibroglia, so that the method cannot be depended upon outside of the central nervous system.

In differentiation, after most of the toluol is wiped off the slide, the section is flooded with the pure oil of cloves. The slide is then agitated in the differentiating solution and the degree of decolorization is controlled under the microscope. This should be carried to the point where the blood vessels stand out as orange rings. If the decolorization is too rapid, more of the oil of cloves may be added to the differentiating solution. The affinity of the tissues for the dye varies somewhat, the protoplasmic neuroglia of the cortex retaining the stain strongly.

If the tissue has been mordanted for over a week previous to staining, there is danger that the myelin sheaths will take the stain. Occasionally this may occur in old formalin-fixed tissue.

It is important that the staining solution be prepared carefully. The stock solution, if tightly stoppered, will keep indefinitely. This method has its greatest use in tumor work, and although experience is necessary to obtain the best results, the method is reliable and the preparations are excellent for

photography. The stain is not permanent and tends to fade. It should not be exposed to direct sunlight or heat.

Holzer Glia-Fiber Stain.—With this method, frozen paraffin, or celloidin sections of formalin-fixed tissue may be used. Alcohol-fixed tissue may be used, but is not recommended. The method is particularly adapted to the study of general neuropathologic material, although useful preparations of tumor tissue can be obtained. The method is one of the best for glia fibers.

(a) *Frozen Sections.*

1. Place sections cut at 15 microns in 50 per cent alcohol and allow to remain for one to two hours.

2. Draw on slide covered with egg albumen-glycerin. Blot dry with filter paper.

3. Wash three times with phosphomolybdic acid solution:

0.5% phosphomolybdic acid, aqueous	1 part
96% alcohol	3 parts

The individual components keep well, but the mixture should be prepared fresh.

4. Blot three times with filter paper soaked in the following alcohol-chloroform mixture:

Absolute alcohol	2 c.c.
Chloroform	8 c.c.
Prepare fresh.	

Firm pressure will prevent the sections from coming off the slides.

5. Cover sections with this same alcohol-chloroform mixture until the sections clear. Do not allow drying to take place.

6. Add crystal violet solution directly to the above.

Crystal violet	1 gm.
Absolute alcohol	2 c.c.
Chloroform	8 c.c.
Filter. Solution keeps well.	

7. Add quickly a 10 per cent solution (aqueous) of potassium bromide by drops until the green sheen which forms disappears. Blot dry with filter paper.

8. Differentiate in the following solution:

Aniline oil	4 c.c.
Chloroform	6 c.c.
1% aqueous acetic acid	1 drop
Filter. Prepare fresh before use.	

It is necessary to control the differentiation under the microscope. When the desired decolorization is attained, flood the slide with xylol and mount in balsam.

It is necessary to work quite rapidly throughout the entire procedure. Because of this, it is convenient to keep the various reagents in small dropper bottles, pouring the solutions over the slides. Coplin jars cannot be used

because of the rapidity with which most of the solutions evaporate. When examining the sections under the microscope, wash out the differentiating solution with xylol lest the decolorization be carried too far. It may be necessary to repeat the process several times before the correct degree of decolorization is obtained.

(b) *Paraffin Sections.*

1. Place deparaffinized sections for three minutes in the following freshly prepared mixture:

0.5% phosphomolybdic acid, aqueous	10 c.c.
96% alcohol	20 c.c.

2. Drain off the fluid and cover section with:

Absolute alcohol	2 c.c.
Chloroform	8 c.c.
Prepare fresh.	

3. While still wet, cover with the following staining mixture and allow to remain so for thirty seconds:

Crystal violet	0.5 gm.
Absolute alcohol	2.0 c.c.
Chloroform	8.0 c.c.
Filter. This solution keeps well.	

4. Replace dye with 10 per cent aqueous potassium bromide solution and wash in this same solution for one minute.

5. Differentiate for thirty seconds in:

Aniline oil	6 c.c.
Chloroform	9 c.c.
Ammonia, 25%	1 drop
Prepare fresh.	

6. Wash in xylol. If the section is overstained, repeat differentiation, controlling under the microscope. Mount in balsam after washing thoroughly in xylol.

(c) *Celloidin Sections.*

Before staining, celloidin sections should be allowed to remain overnight in methyl alcohol. Draw on to glass slides directly from this and blot dry. Place sections for a short time in 50 per cent alcohol; dry and proceed as in the method outlined for frozen sections. Celloidin sections may also be mounted on glass slides before treatment with methyl alcohol. A mixture of equal parts of absolute alcohol and ether may be used also to remove the celloidin.

With the frozen sections and the paraffin-embedded tissue the background should be practically colorless. Where celloidin sections have been used the background is usually colored a light blue, but often not uniformly so. Glial cells stain clearly. The fibers and nuclei are colored a deep shade of blue,

the depth of the staining depending upon the degree of decolorization of the tissue. In celloidin sections there may be a tendency for the perivascular connective tissue to take the dye slightly deeper than the background but never enough to interfere with the recognition of the glial fibers. Myelin sheaths, reticulum, axis cylinders, and ganglion cells are not stained. The best results are obtained on frozen and paraffin sections, and these should be used in preference to celloidin sections whenever possible.

Once started, the procedure must be carried through to completion. Precipitates can be avoided by working rapidly and using fresh solutions which are carefully filtered. With the exception of the dye and the potassium bromide solution, all solutions should be prepared fresh.

Warkany's modification, in which frozen sections are used, consists of placing the sections for one to two minutes in equal parts of 1 per cent phosphomolybdic acid and 95 per cent alcohol, washing in a mixture of 4 parts of chloroform and 1 part of 95 per cent alcohol and staining for a few seconds in a mixture of 8 c.c. chloroform, 0.5 gm. crystal violet and 2 c.c. 95 per cent alcohol. The sections are then washed in 95 per cent alcohol for a short time and differentiated in two changes of aniline oil. This method has the advantage of being simpler and less time-consuming than the original Holzer method, but gives results which are less consistent.

Alzheimer-Mann Stain for Neuroglia.—This stain, although primarily intended for the study of neuroglia, is important in that it allows the study of both myelin sheaths and axis cylinders in the same preparation. The tissue should be fixed in Weigert's glial (secondary) mordant for at least two weeks. Prolonged fixation has no ill effects.

1. Wash the blocks of tissue for twelve to twenty-four hours to remove the excess copper. Shorter periods of washing, while not deleterious to the stain, will not completely remove the excess copper which may injure the edge of the microtome knife.

2. Cut frozen sections at 10 to 15 microns.

3. Mordant in a saturated aqueous solution of phosphomolybdic acid. The sections may remain in this solution for as long as twelve hours, although one hour is usually enough.

4. Wash quickly in two changes of distilled water.

5. Place in Mann's solution for one to two hours:

1% aqueous methyl blue	35 c.c.
1% aqueous eosin	35 c.c.
Distilled water	100 c.c.

6. Wash rapidly in distilled water.

7. Differentiate in 96 per cent alcohol. This takes place quite rapidly. The white matter should be bright red or pink, and the gray matter a light blue. The differentiation can be controlled by the color of the gray matter.

8. Rapidly dehydrate in absolute alcohol, clear in xylol, and mount in balsam.

Normal myelin sheaths are colorless or pink, depending upon the degree of decolorization. Degenerating myelin sheaths tend to assume a deeper shade and may stain bright red. Normal axis cylinders stain a deep blue, while degenerating axis cylinders are a bright red. The cytoplasm of the glial cells is a pale blue color, while neuroglia fibers take a dark blue stain. Amoeboid glia vary in color from a pale to deep blue and often show stained inclusion bodies. Connective tissue stains a dark blue. In the cortex the differentiation is less clear; the nerve cells take a dark blue color and show relatively little detail.

The method is of particular value in studying spinal cord and peripheral nerves, especially for the pathologic forms of glia and the axis cylinders. Mallory states that the change from blue to red staining of the latter takes place as early as forty-eight hours after a lesion, while degeneration as demonstrated by the secondary changes in the myelin sheaths (Marchi method) begin to appear only after about seven days.

Although the best results are obtained upon tissue which has been placed directly into the glia mordant, formalin-fixed tissue which has been placed in the glia mordant and allowed to remain two weeks may also be used. After the frozen sections are cut, they may be stained at once or allowed to remain overnight in the glia mordant. Instead of utilizing a saturated solution of phosphomolybdic acid, Wertham allows the sections to remain for a half hour in a 0.5 per cent solution, after which they are transferred to a 10 per cent solution for three to four hours. The sections should be handled with glass hooks and care should be taken that they lie flat in the various solutions. Beautiful preparations may be obtained by using this method as a counterstain for the Bielschowsky silver method.

Chapter VII

Metallic Glia Stains

Although many of the nonmetallic glia stains give excellent pictures of the glial fibers, few give as clear-cut and detailed staining of the cell bodies and their processes as do the metallic impregnation methods. Although practically all of the original methods require special fixatives, many modifications make possible the use of formalin-fixed tissue. Only those have been selected which give relatively consistent results.

The technique of impregnating the interstitial cells of the central nervous system requires certain care and attention to details which is not usually called for in the staining of other tissues. Although small variations in timing, temperature, and such will often be found necessary to attain the best results by the individual worker, the techniques are more or less standard, and certain factors must be followed closely. The glial cells are particularly susceptible to post-mortem change, and as such the tissue should be fixed as soon after death as possible. All glassware must be chemically clean and washed in distilled water prior to use. Unless otherwise indicated, distilled water is to be used for all solutions. Sections should be handled with small glass rods. At the best, metallic stains are apt to be rather capricious, requiring patience and practice.

Cajal's Gold Sublimate Method.—This method originally called for tissue fixed in formalin-ammonium bromide. Many preliminary treatments for formalin-fixed tissue have been described, so that at the present time excellent results can be obtained on routine laboratory material. Most of these preliminary treatments follow the work of Globus and depend upon the introduction of bromine in some form into the tissue. However, good results can be obtained occasionally by the use of formalin-fixed tissue without previous treatment, particularly if the tissue has not been fixed in the formalin over two days. The method is one of the simplest and one of the most useful for tumor tissue. Frozen sections, 20 to 30 microns, are used. The method, without previous treatment of the tissues, is as follows:

1. Wash sections in distilled water.

2. Stain from four to eighteen hours (overnight usually) in the following mixture:

1% gold chloride (acid brown)	6 c.c.
1% mercuric chloride	25 c.c.
Distilled water	5 c.c.

Prepare fresh before use.

This is enough for 6 to 8 average-sized sections. If the sections are particularly large, only 4 should be stained in this amount of solution.

3. Wash in distilled water.

4. Fix for five to ten minutes in 5 per cent sodium thiosulfate. The sections become quite flexible in this solution.

5. Wash well in distilled water.

6. Transfer to 50 per cent alcohol. Mount on glass slides, blot carefully but firmly, complete dehydration in two changes of absolute alcohol, clear in xylol, and mount in balsam. When completely dehydrated the sections are clear when seen with transmitted light, but appear almost opaque with reflected light. They are purplish red or reddish brown in color. They do not fade.

The microscopic picture depends considerably upon the depth of staining. When the staining has been intense, the glial cells, particularly the astrocytes, appear deep purple to black, and the background is a lavender or light reddish-purple color. The glial fibers are of the same purplish-black color. With less intense staining the background is a light lavender, and the individual fibers and cell processes are more distinctly seen. In some instances a certain amount of nuclear detail is evident. Ganglion cells may be faintly impregnated and the capillaries may be outlined by the perivascular extensions of the astrocytes which take the stain deeply.

The length of time required for complete staining varies considerably. When the sections have assumed a purple color, it is best to remove one and examine it under the microscope. In the wet preparation, with the low-power lens of the microscope, the astrocytes should stand out as deeply stained black stars. The affinity for this stain varies with different tissues, so that the

sections should be watched to prevent overstaining. It is not necessary to keep the staining solution in the dark. With tissue which has been fixed in formalin, especially if the period of fixation has been long, there is a tendency toward granular staining. If the tissue has been in formalin very long, the astrocytes may become refractory to the gold.

The following pretreatment has been suggested by Globus for material which has been hardened in formalin. It may be used for both the Cajal and Hortega stains.

1. Frozen sections at from 15 to 30 microns.
2. Wash quickly in several changes of distilled water.
3. Place in a 10 per cent solution of strong ammonium hydroxide for twenty-four hours at room temperature, or for four hours in an incubator.
4. Rinse rapidly in two changes of distilled water.
5. Place in a 10 per cent solution of concentrated (41 per cent) hydrobromic acid for two to four hours.
6. Wash rapidly in two changes of distilled water to which a few drops of ammonium hydroxide have been added. Proceed with the Cajal or Hortega stain as usual.

If sections are not to be stained at once, they should be left in 2 per cent ammonium bromide to which a few drops of neutral formalin have been added. Tissue which has been fixed in formalin-ammonium bromide or which has been treated with bromine derivatives may require shorter staining periods in the gold bath. Occasionally, excellent results can be obtained by merely allowing the sections to remain overnight or longer in formalin-ammonium bromide in the incubator at 57° C. Penfield suggests the addition of 2 or 3 drops of a 1:1000 aqueous solution of erythrosin to the gold bath to hasten the staining. A similar result can be obtained by adding enough dry erythrosin dye to the gold bath to impart an orange tint. Moderate increases in temperature also favor the staining reaction.

Corten's Modification of the Cajal Gold Sublimate stain was devised for formalin-fixed tissue and gives good results.

1. Frozen sections, 20 to 25 microns, are heated in the following solution until steam arises:

Ammonium bromide	15 gm.
Formol (undiluted and neutral)	100 c.c.
Double distilled water	400 c.c.

Do not boil.

2. Transfer directly without washing into:

Antiformin	3 c.c.
96% alcohol	8 c.c.

3. Wash thoroughly in two changes of double distilled water.

4. Stain for one to two hours or until the sections are brownish red in the following solution:

1% aqueous gold chloride (acid brown)	4 c.c.
5% aqueous mercuric chloride	8 c.c.
Double distilled water	6 c.c.

During the staining, the sections should be kept in the dark in the incubator at 37° C. The staining should be controlled under the microscope but sections should be washed in double distilled water before examination.

5. Wash thoroughly in double distilled water and fix in 5 per cent hypo for fifteen minutes.

6. Wash thoroughly in distilled water, mount on a slide from 50 per cent alcohol, blot firmly but carefully, complete dehydration in absolute alcohol, and clear in xylol. Mount in balsam.

The antiformin should be active and it may be necessary to prepare it fresh. The final results of the stain are those described previously.

Gelatin-embedded material may be used if sections are cut at 10 microns or less. Wertham advises the following gold bath:

1% gold chloride	10 c.c.
5% aqueous mercuric chloride	6 c.c.
Distilled water	4 c.c.

The time required for staining is usually lengthened.

In general, the stain is the simplest and the most reliable of the metallic methods for glial cells.

Kanzler's Modification of Hortega's Method for Microglia.—Of the numerous modifications of the original Hortega method for the silver impregnation of microglia, this one gives good results. Tissue fixed in formalin or formalin-ammonium bromide may be used.

1. Frozen sections, 25 to 30 microns, are received in distilled water and transferred to the following formalin-ammonium bromide mixture in which they are heated until steam arises:

Ammonium bromide	15 gm.
Pure formalin	100 c.c.
Distilled water	400 c.c.
This solution keeps well.	

Allow to cool fifteen minutes or longer. Do not boil.

2. Without washing, place directly into the following alcohol-antiformin bath:

Antiformin	3 c.c.
96% alcohol	8 c.c.
Distilled water	2 c.c.
Prepare fresh before using.	

It is essential that the antiformin be fresh and active. Allow to remain five to eight seconds, or until the white matter becomes apparent. Keep sections in motion during this time.

3. Wash in two changes of distilled water and transfer to the following silver bath:

10% silver nitrate	5 c.c.
10% sodium carbonate	15 c.c.

Dissolve the resultant precipitate by the addition of strong ammonium hydroxide, drop by drop, shaking continuously. Avoid an excess of ammonia. No further water is added. Filter before use. Prepare fresh.

Allow sections to remain eight to fifteen seconds.

4. Transfer without washing to 2 per cent formalin made up with distilled water. Develop quickly, keeping the sections in motion and renewing the solution frequently.

5. Wash freely in distilled water.

6. Tone for ten to twenty minutes in the following gold bath:

1% gold chloride (yellow)	1 to 2 c.c.
Distilled water	10 c.c.

7. Wash and fix in 10 c.c. of a 5 per cent solution of sodium thiosulfate to which 1 gm. of sodium sulfate has been added. The addition of the latter is not absolutely necessary. Allow sections to remain for one minute.

Wash well in distilled water. Mount sections from 50 per cent alcohol, blot firmly, and complete dehydration in absolute alcohol. Clear in creosote carboxylol and mount in balsam.

Microglia is seen as small cells containing an elongated, rod-shaped or triangular nucleus and short, irregular processes. The cells are stained a grayish black and stand out sharply from the light gray background. There is impregnation of the nuclei of the other interstitial cells, but only rarely can the outline of the astrocytes be seen. Axis cylinders, myelin sheaths, neurons, and such are not stained.

Failure to obtain good staining may be due to several factors. The sodium carbonate used in the preparation of the silver bath must be free from impurities. Imperfectly distilled water may account for some failures. The antiformin must be active, and it may be necessary to prepare it fresh by mixing equal parts of 15 per cent sodium hydroxide and 20 per cent sodium hypochlorite. The commercial antiformin may not give good results. It is often necessary for the individual worker to try minor empirical variations in the technique if the method does not succeed the first few times. The pathologic forms of microglia stain more easily than the microglia in normal brain tissue. The beginner will find it helpful to become acquainted with the method through the use of tissue from a case of dementia paralytica where

the microglia cells are increased in size and number, particularly in the frontal lobes. In general, this method gives fairly consistent results, and the completed preparations are excellent for photography.

Penfield's Second Modification for Combined Oligodendroglia and Microglia.—With this modification of Hortega's silver carbonate method there is staining of both the oligodendroglia and microglia cells. It can be applied to formalin or formalin-ammonium bromide fixed tissue.

1. Frozen sections, cut at 20 microns, are received into 1 per cent formalin or distilled water. The former is preferable, since it prevents swelling of the tissue.

2. Place sections in distilled water to which 10 to 15 drops of strong ammonium hydroxide have been added. Cover and allow to remain overnight.

3. Without washing, transfer sections to 5 per cent Globus' hydrobromic acid, and place in incubator at 38° C. for one hour.

40% hydrobromic acid	5 c.c.
Distilled water	95 c.c.

4. Rinse in three changes of distilled water.

5. Mordant sections in 5 per cent sodium carbonate for one hour. They may remain here for five to six hours without ill effect.

6. Pass sections, preferably without washing, into Hortega's weak ammoniacal silver carbonate solution for three to five minutes, or until they turn a smooth gray when transferred to the reducer. At times good results may be obtained by leaving the sections in the silver solution until they turn light brown. The impregnation should be controlled under the microscope. Hortega's weak ammoniacal silver carbonate solution is prepared as follows:

10% silver nitrate	5 c.c.
5% sodium carbonate, pure	20 c.c.

Add sufficient fresh ammonium hydroxide, drop by drop, to just dissolve the precipitate, stirring constantly. Do not add too much ammonia. A fine black precipitate, unlike the voluminous silver carbonate, remains. Filter and dilute to 75 c.c. Keep in a dark bottle.

7. Reduce in 1 per cent formalin, agitating sections constantly. Use fresh formalin for each section.

8. Wash in distilled water and tone in 1:500 gold chloride at room temperature until sections are a smooth gray.

9. Fix in 5 per cent sodium thiosulfate for a few minutes.

10. Wash freely in distilled water and mount on a glass slide from 50 per cent alcohol. Blot, complete dehydration in absolute alcohol, clear with creosote carboxylol, and mount in balsam.

With this method there is staining of the oligodendroglia and microglia with a fair degree of consistency. The two types of cells can be distinguished by their differences in morphology, the oligodendroglia appearing as rela-

tively small cells with large spherical nuclei, scanty cytoplasm, and a few fine processes. The microglia appear as described in the Kanzler stain. Occasionally there is faint impregnation of the astrocytes, especially if the sections have been allowed to remain in the silver bath too long. The background is practically colorless, axis cylinders, neurons, and such not taking the stain. By allowing the sections to remain in the ammonia two nights and lengthening the time of treatment in the silver bath, a more intense impregnation is obtained.

The method works best if the tissue is not too old, although good results can be obtained on old formalin-fixed material. Tissue fixed in formalin for not over two days gives excellent results. As in the Kanzler modification, failure to obtain good impregnation may be due to imperfectly distilled water, impure sodium carbonate, or improperly fixed tissue. Whenever possible, tissue fixed in formalin-ammonium bromide should be used and the silver carbonate solution prepared fresh, although the latter keeps for several weeks when tightly stoppered and stored in an amber bottle in the dark.

Glia Stains on Paraffin Sections.—Practically all of the selective metallic methods for the demonstration of glia cells require the use of frozen sections. Occasionally, however, unembedded tissue is not available and it becomes necessary to use paraffin sections. Numerous methods have been devised for the silver impregnation of paraffined tissue, none of which are very satisfactory. The methods are usually not very reliable and rarely are they selective, impregnating nerve fibers, glia cells, glia fibers, and connective tissue at the same time. They are useful, nevertheless, if only as a means of obtaining a general survey for comparison with the more specific stains.

Wilder's Method for Glia and Nerve Fibers.—Paraffined sections, cut at 8 to 10 microns, are brought to distilled water. Tissue should be fixed in formalin.

1. Place in 34 per cent hydrobromic acid for thirty minutes.
2. Wash in distilled water for ten to twenty seconds.
3. Flood the slide for five seconds with 1 per cent aqueous uranium nitrate solution, sodium free. It may be necessary to use a shorter time.
4. Wash in distilled water for ten to twenty seconds.
5. Place in silver diamino-hydroxide for twenty seconds.

   To 5 c.c. of 10.2% silver nitrate, add strong ammonium hydroxide until the precipitate which forms just redissolves. Add 5 c.c. of 3.1% sodium hydroxide and dissolve the precipitate which forms by adding ammonium hydroxide, drop by drop. Make up to 50 c.c. with distilled water. Prepare fresh just before use.

6. Wash briefly in distilled water.
7. Agitate each slide separately in the following reducing solution until the brown cloud is no longer given off:

1% uranium nitrate	1.5 c.c.
40% formalin, neutralized with magnesium carbonate	0.5 c.c.
Distilled water	50.0 c.c.

8. Wash in distilled water.

9. Counterstain with eosin. Dehydrate in alcohol, clear in xylol, and mount in balsam. Hematoxylin may be used but must be blued in tap water.

The ganglion cells, nerve fibers, and glia cells and their processes are stained black. The astrocytes and spongioblasts are well impregnated. Tissue fixed twenty-four hours after death shows impregnation of the nerve fibers and fibrous astrocytes, but the processes of the protoplasmic astrocytes and the oligodendroglia cells cannot be shown when more than six hours have elapsed between the time of death and fixation. As in other metallic methods, if fixation has not been prompt, the staining is apt to be granular.

The uranium nitrate and silver nitrate solutions keep indefinitely. The silver nitrate should be stored in a dark bottle out of the light. The silver diamino-hydroxide, if stored in a tightly stoppered amber-colored bottle, will keep for a week.

Fincher's Silver Carbonate Method.—This method is particularly well adapted for tumor tissue. It utilizes Hortega's silver lithium carbonate. Thin paraffin sections are brought to distilled water.

1. Mordant in the following solution at 60° C. until sections are colored yellow:

2% silver nitrate	30 c.c.
Pyridine	10 drops
Alcohol	15 drops

2. Dip in 95 per cent alcohol. Wash sections in water and impregnate in the following silver bath:

Silver carbonate, lithium	30 c.c.
Pyridine	10 drops
Alcohol	15 drops

Place in the incubator at 60° C. until sections are amber.

3. Wash rapidly in 95 per cent alcohol.

4. Reduce in 10 per cent formalin.

5. Wash in distilled water.

6. Tone in 1:500 gold chloride solution in the incubator at 60° C. until sections are a grayish-red color.

7. Wash in distilled water and fix in 5 per cent sodium hyposulfite solution.

8. Wash in distilled water, dehydrate in alcohol, clear in creosote carboxylol, and mount in balsam.

The neural and glial elements are stained a pinkish-red color in contrast to the more heavily impregnated fibroblastic elements which assume a dark brown color. Astrocytes and spongioblasts are well stained.

Zenker-fixed tissue may be used but should be passed first through Gram's iodine, followed by 5 per cent sodium thiosulfate to remove the mercuric chloride crystals. Tissue which has been fixed in Kaiserling's fluid for museum purposes may also be stained. An excess of silver in the tissue may be removed by allowing the sections to remain overnight in xylol to which a few drops of pyridine have been added.

Hortega's silver carbonate (lithium) is prepared as follows:

10% silver nitrate	5 c.c.
0.5% lithium carbonate	20 c.c.

Add strong ammonium hydroxide, drop by drop, until the precipitate is just dissolved. The solution should be stirred continuously and care taken to avoid an excess of ammonia. A small amount of fine black precipitate will remain and may be filtered off. Make up to 75 c.c. with distilled water and store in firmly stoppered brown bottle. The solution keeps for several weeks.

(To be continued.)

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TYPHOID VACCINATION, A Study of Oral, as Measured by Blood Serum Agglutinins, Crimm, P. D., and Short, D. M. Am. J. M. Sc. **196**: 826, 1938.

Using typhoid-mixed vaccine, 100 cases were vaccinated by the oral and a like number by the subcutaneous method. Blood serum agglutinins were determined periodically.

Thirty-three per cent of the cases which received the oral typhoid-mixed vaccine had mild reactions, none of which were incapacitating.

Significant agglutinin titers with oral typhoid vaccine are present within sixty hours. The agglutinin titration of both methods is essentially comparable after an interval of from two to five weeks. Significant titers persist for six months or longer following oral vaccine.

Those cases failing to show agglutinins five weeks following vaccination were 24 per cent for the subcutaneous, and 8 per cent for the oral cases. Thus, the failures are three times as numerous with the subcutaneous as with the oral method.

The authors conclude, from the extensive clinical reports of others and from the present laboratory results, that the oral method is as efficacious as the subcutaneous method of mixed typhoid vaccination.

MALARIA, New Method of Staining Thick Films, Simons, H. Bull. Soc. path. exot. **31**: 100, 1938.

For the purpose of rapid diagnosis of blood parasites the author advocates the use of the following mixture, which dehemoglobinizes the red blood cells by virtue of the saponin, and stains the parasites and leucocytes by virtue of the methylene blue.

Distilled water	300 c.c.
Methylene blue	0.6 gm.
Sodium chloride	1.8 gm.
Sodium citrate	3.0 gm.
Saponin	2.0 gm.
Formol (15 per cent)	12 c.c.

The mixture is poured over a thin or thick smear and allowed to act for one or two minutes. In the case of thick films the stain is poured off and fresh stain is applied. According to the thickness of the film, this is repeated three to five times. When staining is complete, the stain is poured off and a cover glass is applied, examination of the film being made in the small quantity of stain remaining under the cover glass. There being little danger of the thick film floating off the slide, drying in the first place may be conducted rapidly by repeated gentle warming in a flame and blowing. The whole process of preparation and staining of a thick film does not occupy more than three or four minutes—a considerable saving of time when rapid diagnosis is necessary.

ERYTHROCYTES, Red Blood Cell Values in Adolescence, Mugrage, E. R., and Andresen, M. I. Am. J. Dis. Child. **56**: 997, 1938.

The quantity of hemoglobin, the number of red blood cells, and the volume of packed cells were determined accurately on samples of venous blood from 80 boys and 80 girls, between the ages of 13 and 21 years. The values obtained are compared with a series of 640

similar determinations on the blood of infants, children, and adults. From the results of the study, the following conclusions may be drawn:

1. Averages for boys are almost identical with those for girls at 13 years. These gradually increase to the adult level for men at 17 years. Averages for girls show little variation from the level for women during the adolescent period.

2. In the adolescent as well as in the adult, the average volume of the individual red blood cell of the male is smaller than that of the female. Values for adolescents of both sexes are slightly higher than those for adults.

3. The corpuscular hemoglobin concentration remains at the same level throughout adolescence as in other age periods.

DYSENTERY: Specificity of Agglutination Reaction for S. dysenteriae, Mackie, T. T. Arch. Int. Med. **62**: 783, 1938.

Prolonged serologic and cultural studies indicate the necessity for cautious interpretation of the agglutination reaction in cases of chronic inflammatory disease of the colon. The author has repeatedly found agglutinins for *S. dysenteriae* present at titers commonly considered to establish the diagnosis unaccompanied by cultural evidence of homologous infection. Conversely, no agglutination reactions have been observed in cases of proved infection. Marked and unaccountable variations of agglutinin titer are the rule in the course of repeated determinations. It is a striking fact that the majority of the sera which gave a high titer were obtained from patients who consistently showed sterile culture for *S. dysenteriae*. These observations suggest that agglutinins for *S. dysenteriae* may develop in response to nonspecific heterologous stimuli.

LEAD HAZARD, Observations on Men Exposed to, and an Original Method for Detection of Polychromasia, Windsor-McLean, L. A. M. J. Australia **2**: 367, 1938.

Ordinary thin blood films are made on clean, thin (1.0 mm. or less) microscopic slides in the ordinary way. After drying, the films are fixed by running pure methyl alcohol on the slide, film up, and allowing it to remain for two minutes. The film is allowed to dry in the air, and then Seller's stain is run on and allowed to remain on the film for forty-five seconds. The stain is then washed off with running tap water, and the washing continued for one minute. The film should then be very pale green in color. It is allowed to dry in the air and is then examined by the dark-field method.

FREI TEST, Evaluation of, With Mouse Brain Antigen, Reider, R. F., and Canizares, O. Arch. Dermat. & Syph. **38**: 628, 1938.

For 116 patients with lymphogranuloma venereum and 204 control subjects, 398 tests were made with mouse brain antigen, 398 with emulsion of normal mouse brain, and 124 with antigen prepared from pus of human patients.

Criteria are offered for interpretation of the test with mouse brain antigen.

Of patients with lymphogranuloma venereum, 90 per cent gave positive, 9 per cent negative, and 1 per cent doubtful reactions to mouse brain antigen. Most patients giving a negative reaction had the disease in an early stage and later gave a positive reaction.

Of the control subjects 93 per cent gave negative, 5 per cent positive, and 2 per cent doubtful reactions to mouse brain antigen.

One per cent of persons tested showed a strong hypersensitivity to mouse brain tissue, the reaction to emulsion of normal mouse brain and to mouse brain antigen remaining intense for more than a week.

The optimum time to read the result of the Frei test is between the third and the fifth days, preferably the fourth day.

A reaction to mouse brain antigen on the fourth day consisting of a papule more than 6 mm. in diameter, accompanied with a much less intense reaction to emulsion of normal mouse brain, may be considered positive. The reaction should remain this size or become larger for at least one week.

There is usually a reaction to mouse brain antigen and to emulsion of normal mouse brain during the first two days, which then disappears. This is a nonspecific reaction.

In the interpretation of borderline reactions (6 to 7.5 gm.) to mouse brain antigen, the size of the corresponding reaction to emulsion of normal mouse brain should be taken into consideration. A small percentage of reactions are doubtful and cannot be interpreted. The test should be repeated in cases in which such a reaction appears.

Comparison of mouse brain antigen with that prepared from the pus of human patients shows that both give approximately equal reactions.

Mouse brain antigen is suitable for use in the diagnosis of lymphogranuloma venereum when the reaction to it is properly interpreted.

TUBERCULOSIS, Effect of, on Serological Reactions for Syphilis, Parran, T., and Emerson, K. Am. Rev. Tubere. **39**: 1, 1939.

Inasmuch as with the present serologic tests for syphilis both typical and atypical false-doubtful and false-positive results are found in sera from tuberculous donors, it is evident that tuberculous toxemia may contribute a confusing factor to syphilis serology. It should not, however, present a major problem in the clinical interpretation of results obtained with carefully conducted serodiagnostic procedures.

LEUKEMOID REACTIONS of the Myeloid Type, Heck, F. J., and Hall, B. E. J. A. M. A. **112**: 95, 1939.

The importance of differentiating leucemoid reactions from the leucemias is obvious. In most cases this differentiation can be made on the basis of the clinical observations. The hematologic picture may or may not be of additional aid in diagnosis. The total leucocyte count is of relatively little or no diagnostic significance unless it is in excess of 100,000 cells per c.mm. blood. Leucocyte counts above this are observed rarely with conditions other than the chronic forms of leucemia. It should be remembered that many patients with leucemia, including the chronic variety, have total leucocyte counts which fall either within or below the range for normal persons. In a study previously reported, approximately 40 per cent of all patients with acute leucemia and 10 per cent of all patients with chronic leucemia seen at the Mayo Clinic from 1928 to 1933, inclusive, had leucocyte counts below 10,000 cells per c.mm.

Summary.—Leucemoid reactions occur commonly with infections, with blood dyscrasias and diseases of the reticulo-endothelial system, with diseases in which there is invasion and irritation of the bone marrow (as in metastasis to bone), with conditions in which there is an increased demand on the bone marrow (such as severe and sudden loss of blood), and with chemical poisoning. Occasionally cases are encountered in which no satisfactory explanation for the leucemoid reaction can be made.

TISSUE: Preparation of Plaster of Paris Embedding Boxes, Solberg, A. N. Stain Technol. **14**: 27, 1939.

Cut pieces of paraffin of the size you desire the blocks. Bevel the paraffin block about 15° so that the bottom will be smaller than the top to allow the paraffin blocks to slip out when shrunk by cooling. Seal the block, wide side down, onto a copper plate or piece of cardboard, and place around it two embedding angles about ⅛ inch from the widest part of the paraffin block. Coat the paraffin block with a thin layer of vaseline. Mix enough plaster of Paris and water to fill the mold, and pour the mixture into the mold while still very wet. Allow the plaster of Paris to dry for an hour or more. Remove the embedding angles, and when the plaster of Paris seems quite dry, lift the box off the paraffin mold. When dry, trim the box with a knife and sandpaper to the desired shape and thickness. It is advisable to leave the wall quite thick to prevent breakage.

To use the box: Soak it for a moment in the water to be used for cooling. Pick it out and pour out the water. Holding the box between the thumb and forefinger, fill the box with melted paraffin using a medicine dropper. Drop the tissue into the melted paraffin.

Orient tissue as desired with a warm needle or forceps. Insert a previously prepared slip of paper with identification marks on it into the melted paraffin at one end of the box. Hold the box in the cold water to cool and blow gently on the surface until a thin film has congealed. Carefully lower the box under water and in a few seconds the paraffin will shrink from the sides of the box, allowing the block to float free. The box may be used again immediately, but one can work faster by using two boxes alternately.

HYPERGLYCEMIA, A New Interpretation of, in Obese, Middle-Aged Persons, Newburgh, L. H., and Conn, J. W. J. A. M. A. **112**: 7, 1939.

A statistical analysis of spontaneous glycosuria associated with delayed disposal of ingested dextrose indicates that approximately half of the patients are obese. These obese glycosuric patients are, with few exceptions, more than 30 years of age.

After the weight of these patients has been reduced to normal by simple underfeeding, they remain aglycosuric, do not become hyperglycemic when they are placed on maintenance diets containing 300 gm. of carbohydrate, and exhibit normal dextrose tolerance curves.

There is an occasional exception to this rule, but more than 90 per cent of the patients respond in this manner.

Recurrence of the obesity is capable of reproducing the hyperglycemia and the delayed utilization of dextrose. Subsequent reduction of weight again corrects the disturbance in the metabolism of carbohydrate.

It has been demonstrated that the majority of persons who have been obese for many years show delayed utilization of carbohydrate. Since the majority respond in this way to adiposity and again dispose of carbohydrate normally when the excessive weight has been removed, this phenomenon must be regarded as being the type response of the previously normal mechanism carbohydrate metabolism to the overload of obesity.

It is suggested that the occurrence of the hyperglycemia and glycosuria in such persons depends on the excessive accumulation of fat in the liver, with a resulting impairment in its capacity to lay down glycogen at the normally rapid rate.

The studies described establish a clinical entity in which obesity is the principal abnormality and hyperglycemia is a secondary phenomenon.

AGGLUTINATION, Salt Optima in, Platt, A. E. Australian J. Exper. Biol. & M. Sc. **16**: 275, 1938.

Using a smooth strain of *Brucella abortus* and a corresponding antiserum obtained from an infected cow, it was found that the antigen and antibody optimal ratios remained constant for all salt concentrations between 2M/7 and M/56. Concentrations of salt above 2M/7 were not used, and those below M/56 failed to agglutinate the sensitized organisms.

The optimal salt concentration for any agglutinating system depends upon (a) the ratio of antigen to antibody, (b) the concentration of organisms per unit volume of mixture, and (c) the effect of the salt on the cohesive force between the organisms.

GOLD SOLS, Variations in the Behavior of Paretic Cerebrospinal Fluids With Different Types of, Pennycuick, S. W., Woolcock, C. E., and Cowan, R. J. Australian J. Exper. Biol. & M. Sc. **16**: 317, 1938.

The oxalate-reduced gold sol can be used in the Lange test if a buffering agent is added to the solution either before or after the reduction of the gold salt. In general, however, it is not as reliable as the formaldehyde-reduced sol.

The phosphorus-reduced gold sol cannot be used with any degree of precision.

The Bredig gold sol can be adapted for use, but its reactions are variable and its preparation is inconvenient.

A trace of stannic chloride when added to distilled water facilitates the formation of gold sols, but such sols, while they are suitable for paretic cerebrospinal fluids, show slight irregularities with normal fluids.

PREGNANCY, Regulation of Level of Calcium in the Serum During, Bodansky, M., and Duff, V. B. J. A. M. A. 112: 223, 1939.

Within certain limits (not lower than 8.5 mg.) the depression of calcium in the serum observed in late pregnancy may be considered a normal condition, conducive perhaps to a greater economy of utilization of calcium derived from food and maternal bodily reserves.

Severe hypocalcemia during pregnancy may reflect either parathyroid deficiency or marked nutritional deficiency. Both are comparatively rare in ordinary clinical experience. The importance of the parathyroid glands in the regulation of the calcium content becomes especially impressive in the parathyroid-deficient pregnant animal at term.

Abnormalities in fetal skeletal development occur if hypocalcemia is severe, as shown by the results in experimental parathyroid deficiency and in clinical osteomalacia. The somewhat elevated calcium level in the fetal circulation may, therefore, be looked upon as normal for fetal development.

Nutritional, hormonic, and seasonal factors influence the maternal calcium level. The influence of season may be only partly related to the amount of available sunshine.

It is possible that in pregnacy the maintenance of a subnormal calcium level in the maternal serum, as judged by nonpregnancy standards, despite the augmentation in parathyroid function, is dependent on an intrinsic calcium-depressing factor. The maintenance of a relative elevation of calcium in the fetal serum may also indicate the participation of some factor in the fetal organism independent of the fetal parathyroid glands. The rise in the maternal calcium level after parturition and the decline in that of the newborn infant suggest that these factors may reside in the maternal and fetal portions of the placenta, respectively, each playing a distinct and important role in the regulation of the maternal and the fetal calcium levels. The decline in calcium content observed in the newborn may also be conditioned by the sudden withdrawal of the labile calcium reserve of the fetal placenta.

MENINGOCOCCIC INFECTIONS, Sulfanilamide in the Treatment of, Waghelstein, J. M. J. A. M. A. 111: 2172, 1938.

There were 106 patients with meningococcic infection treated at Sydenham Hospital with 19 deaths, a fatality rate of 18 per cent. The case fatality rate in the adequately treated group was 12 per cent.

When sulfanilamide alone was used, the fatality rate was 15 per cent. In the adequately treated cases, the fatality rate was 12 per cent.

The combined method of treatment was used in 34 cases, with a case fatality rate of 24 per cent. The fatality rate in the adequately treated group was 13 per cent. Among the last 26 patients treated in this manner there were 3 deaths, 2 of which occurred within twenty-four hours.

The spinal fluid cultures were more consistently sterile after from twelve to twenty-four hours of treatment in those patients in which sulfanilamide was taken every four hours by mouth or by nasal catheter, rather than when given every twelve hours by hypodermoclysis.

The number of lumbar punctures in the treatment of meningitis has been markedly reduced.

The complications due to sulfanilamide therapy were infrequent and very mild. The number of recurrences were reduced. Those complications due to meningococcic infections were not significantly reduced in occurrence.

Since the introduction of sulfanilamide in the treatment of meningococcic infection, there has been a definite decrease in the number of deaths, not only in the adequately treated, but also in those fulminating cases which usually terminate fatally within twenty-four hours. The discomfort suffered by the patient directly due to the treatment is lessened owing to the simplification of the treatment, and the hospital stay has been notably reduced.